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REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART I.

LONDON:

HARRISON AND SONS, ST. MARTIN'S LANE,
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INTRODUCTION.

The Mediterranean Fever Commission had its origin in a letter from Mr. Secretary Lyttelton, dated January 25, 1904, addressed to the Royal Society, in which he states that his attention has recently been called to the prevalence of Mediterranean fever in Malta among the Naval and Military forces, as well as the civil population.

It accordingly appeared to him to be desirable that the investigation of this fever should be taken in hand, and he addressed a despatch to the Governor of Malta proposing the appointment of a joint Commission representing the Army, the Navy, and the Civil Government.

He enclosed a copy of a despatch from the Governor in reply, entirely concurring in the proposed appointment of a joint Commission. The War Office and Admiralty also expressed their concurrence in the proposal.

Mr. Secretary Lyttelton then went on to say that the War Office, the Admiralty, and the Civil Government desired to secure for this Commission the assistance of the Royal Society, and asked whether the Society would be willing to appoint an Advisory Board of experts in this country for the purpose of supervising the investigations.

In reply to this letter the Royal Society wrote, in February, 1904, consenting to nominate a Committee to direct the investigations, on the understanding that the selection of the investigators should be placed in the hands of the Royal Society.

A Sub-Committee of the Tropical Diseases Committee was accordingly formed, consisting of Colonel Bruce, R.A.M.C., Chairman, Fleet Surgeon Bassett-Smith, R.N., Dr. Klein, Dr. C. J. Martin, and Dr. Sidney Martin.

As it was desirable to begin the investigations with as little delay as possible, the Sub-Committee at once appointed Major Horrocks, R.A.M.C., Staff-Surgeon Shaw, R.N., and Dr. Zammit, Board of Health, Malta, as members of the Commission, and Colonel Bruce was requested to proceed to Malta to assist them in commencing the work. Colonel Bruce arrived in Malta on June 13, where he met the Commission, and work was at once begun. He remained in Malta until July 14, when he left for England. Dr. Johnstone, whose services were lent by the Local Government Board, on the application of the Royal Society, joined the Commission on June 30.

The best thanks of the Commission are due to the Governor, General Sir C. M. Clarke, G.C.B., and to the Lieutenant-Governor, the Hon. E. M. Merewether, C.M.G., for their courtesy and invaluable aid.

The following reports have been received, up to the present date, from the members of the Commission, and also one from Staff-Surgeon Gilmour, R.N., Bighi Hospital, Malta, who kindly placed his spare time at the service of the Commission.

1.

ON THE DURATION OF LIFE OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE HUMAN BODY.

(Experiments made at Gibraltar.)

By Major W. H. HORROCKS, R.A.M.C., Member of Mediterranean Fever Commission.

(Received July 14, 1904.)

The small size and slow growth of the *Micrococcus melitensis* render the study of its saprophytic existence by no means an easy matter. In the hope of devising a medium which would simplify the isolation of the *Micrococcus* from a mixture of microbes, a careful study of its cultural characteristics on all modern media of an exact reaction was first made. It was thought that the degree of fermentation or non-fermentation of the various sugars might assist in attaining the desired differentiation. The results of the tests are shown in the following table:—

Cultural Characteristics.

| | |
|---|--|
| <i>Glucose peptone</i> , 1 per cent. . . . | Growth. Neither acid nor gas produced. |
| <i>Lactose peptone</i> , " " . . . | " " " |
| <i>Saccharose peptone</i> , 1 per cent. . . | " " " |
| <i>Starch peptone</i> , 1 per cent. . . . | " " " |
| <i>Litmus milk</i> | No clotting observed; at the end of 3 weeks the medium was found to have a distinctly alkaline reaction. |
| <i>Peptone and salt solution</i> | On the addition of a nitrite and pure sulphuric acid, the nitroso-indol reaction was never obtained. |
| <i>Broth</i> | Diffuse growth without any surface pellicle. After some days the broth cleared somewhat, and a deposit formed on the sides and at the bottom of the tube. |
| <i>Agar slope</i> | Greyish-white moist growth; discrete colonies, circular and transparent, resembling those of the Gram-staining streptococci found in fæces and urine. When the cultures are old, the growth often acquires a yellowish-brown colour. |

| | |
|---|--|
| <i>Proskauer and Capaldi's media</i> | No. I. No growth. No. II. Growth, but no change appeared in the reaction of the medium. |
| <i>Neutral red</i> | Unchanged after 48 hours at 37° C.; after 5 days' incubation a yellow colour appeared at the surface. |
| <i>Potato</i> | Moist transparent film appeared, and on scraping the surface a copious growth was obtained. The formation of chains being very marked. The reaction of the potato was made faintly alkaline by the addition of sodium carbonate, and on planting out on the surface a distinct yellowish coloured growth was obtained. |
| <i>MacConkey's bile salt broth</i> ... | Growth; reaction unchanged. |
| <i>Nitrate broth</i> | Growth, but no reduction of the nitrates occurred. |
| <i>Gelatine stab and slope</i> (22° C.) | Growth extremely slow; no liquefaction of the medium. |
| <i>Agar stab</i> (37° C) | Diffuse growth. |
| <i>Anaërobiosis</i> | Growth, but more feeble than under aerobic conditions. |
| <i>Morphology</i> | Very small cocci, appearing as diplococci and short chains; occasionally chains of twelve to fourteen cocci were observed. |

The failure of the *M. melitensis* to ferment glucose, and its power of rendering milk alkaline are very important cultural reactions. The Gram-staining streptococci, isolated from sewage, urine, fæces, cases of erysipelas, and from septic throats, all ferment glucose; the amount of acid produced, however, is a variable quantity. In glucose agar media, tinted with litmus, the Gram-staining streptococci produce colonies varying in tint from a rose red to a bright red, but the colonies of the *M. melitensis* are always blue, and after a few days' incubation the colour deepens in tint.

The gelatine, broth, agar, and peptone media, were made with a reaction of + 10 (Eyre's scale), and as a rule distinct growth was not observed until the 2nd or 3rd day after planting out, incubation being at 37° C.

Several observers having stated that the *M. melitensis* grew best on media having an alkaline reaction; batches were prepared having reactions: - 15, - 10, neutral, + 10, + 15 (Eyre's scale). Approximately, the same amount of culture was planted out, and it was found that the quickest and most copious growth was obtained on the + 10 medium; on the - 10 and - 15 there was practically no growth.

Having determined the most favourable reaction, trials were made to see if a medium could not be obtained on which the *M. melitensis* would grow in 24 hours. Bearing in mind the favourable effect of nutrose on the growth of *B. typhosus*, a 1-per-cent. nutrose agar, + 10,

was prepared, and on this a marked growth of *M. melitensis* occurred in 16 hours. A similar vigorous growth was obtained in nutrose broth.

The study of the cultural reactions having shown that the *M. melitensis* did not ferment glucose, it appeared that the addition of this sugar to the nutrose medium, tinted with litmus, would be of great service when isolating the organism from a mixed culture. As previously stated, the Gram-staining streptococci, which occur in urine and fæces, ferment glucose, forming enough acid to change the blue medium to a rose tint, and as the colonies of these organisms have much the same transparent appearance as those of *M. melitensis* on nutrose agar, the use of the glucose litmus medium enables a separation to be readily made, and saves much time when investigating plate cultures.

Trials were then made with the *M. melitensis* added to non-sterile water and soil, and it was found that the organism could be readily isolated when it was present in considerable quantity; when, however, only a few cocci were present, there was a marked tendency for the water and soil organisms to grow over the plate, the nutrose evidently accelerating the growth of these organisms. Accordingly, attempts were made to restrain the growth of these organisms by the addition of sodium taurocholate, carbolic acid, malachite green, etc.

A medium containing 0.5 per cent. sodium taurocholate, 1 per cent. peptone and 0.5 per cent. salt was prepared, and the tubes inoculated with *M. melitensis*, urine, soil, and water respectively. The results are shown in the following table:—

| | 24 hours. | 48 hours. | 72 hours. | 96 hours. |
|------------------------------------|-----------|-----------|-----------|-----------|
| Tube 1. <i>M. melitensis</i> | ± | ± | + | + |
| Tube 2. One loop urine | ± | ± | + | + |
| Tube 3. One loop of soil | ± | ± | + | + |

Note.—±, feeble growth; +, good growth; —, no growth.

The growth which appeared in Tube 1, after 48 hours' incubation, was planted out on nutrose agar, and the *M. melitensis* recovered after 3 days' incubation at 37° C.

This experiment showed that, while the sodium taurocholate restrained the growth of the microbes in soil and urine, it had also a marked inhibiting effect on the growth of the *M. melitensis*.

The addition of nutrose to the taurocholate medium was then tried, with the following result:—

| | 24 hours. | 48 hours. | 72 hours. |
|------------------------------------|-----------|-----------|-----------|
| Tube 1. <i>M. melitensis</i> | ± | + | + |
| Tube 2. One loop of urine ... | + | + | ++ |
| Tube 3. One loop of soil | ± | + | + |

The growth in Tube 1, which appeared in 48 hours, was planted out on nutrose agar, and the *M. melitensis* recovered after 48 hours' incubation at 37° C.

The addition of the nutrose caused a more vigorous growth of the *M. melitensis*, but unfortunately the growth of the bacteria in urine was enhanced more than that of the *M. melitensis*. The results with these media when grown at 37° C. being unsatisfactory, the temperature of incubation was raised to 42° C. in the hope that it might cause a more satisfactory separation. Hughes, in his book on Mediterranean fever, stated that the *M. melitensis* would not grow at 42° C., so a preliminary planting out on ordinary agar and nutrose agar was tried. The results were as follows:—

| | 24 hours. | 48 hours. | 72 hours. | 96 hours. | 5 days. |
|---------------------------|-----------|-----------|-----------|-----------|---------|
| Ordinary agar (+10) | — | — | — | ± | ± |
| Nutrose agar (+10) | ± | ± | ± | + | + |

Temperature of incubation, 42° C.

On ordinary agar the growth was much delayed and feeble at the end of 5 days, but on nutrose agar a good growth was obtained after 72 hours.

Nutrose, sodium taurocholate peptone tubes were now inoculated with soil, urine, tap-water and *M. melitensis*. Incubation 42° C.

| | 24 hours. | 48 hours. | 72 hours. | 96 hours. |
|---------------------------------------|-----------|-----------|-----------|-----------|
| Tube 1. One c.c. tap-water .. | — | ± | ± | + |
| Tube 2. One loop soil | — | ± | + | ++ |
| Tube 3. One of urine | ± | + | + | ++ |
| Tube 4. One of <i>M. melitensis</i> . | ± | ± | ± | + |

The results were again disappointing; the method would be of very little use in regard to urine investigation, but might render some assistance when working with inoculated water supplies.

Malachite green, krystal violet, etc., being credited with the power of restraining the growth of saprophytes, the former salt was selected for experiment.

The powder was dissolved in distilled water and the solution added to +10 broth, so as to make dilutions of 0.01 per 1,000, 0.02 per 1,000, 0.05 per 1,000, 0.1 per 1,000, and 0.2 per 1,000. The tubes were incubated at 37° C. for 24 hours, and remaining quite sterile were each inoculated with one loopful of an emulsion of *M. melitensis*. After 24 hours' incubation at 37° C., it was found that there was a good growth of *M. melitensis* in all the tubes except the 0.2 per 1,000. Similar dilutions were then inoculated with urine and soil—the tube containing 0.1 per 1,000 was found to have a marked restraining influence on the growth of the bacteria for a period of 24 hours; but after 48 hours' incubation there was a rapid growth of the bacteria in urine.

Nutrose was then added to the malachite green solution, so that the medium now contained 1 per cent. of nutrose and 0.1 per 1,000 of malachite green.

The tubes were inoculated with an emulsion and incubated at 37° C. After 24 hours it was found that there was a vigorous growth of the *M. melitensis*, but unfortunately, as in the case of the sodium taurocholate, the bacteria in the urine and soil also showed a marked growth. Consequently, it was decided to omit the nutrose from the malachite green broth during the preliminary investigations. A non-sterilised garden soil was inoculated with *M. melitensis* and then planted out in malachite green broth; after 24 hours' incubation at 37° C. a feeble growth occurred, which was stroked over the surface of a series of Petri dishes containing nutrose agar. The plates were incubated at 37° C.; after 24 hours there was practically no growth, but after 48 hours there was a marked growth, and the transparent colonies of the *M. melitensis* were easily detected scattered amongst the larger and opaque colonies produced by the soil organisms. This result was satisfactory, and the procedure appears likely to give useful results.

Carbolic acid was next tried; it was found that the *M. melitensis* grew well in 24 hours in 0.05 per cent. carbolic broth, but this small amount of acid has a very slight restraining influence on the growth of the bacteria in urine and soil, and consequently the *M. melitensis* was always crowded out by the saprophytic bacteria. The amount of carbolic acid was increased to 0.1 per cent., but in this the *M. melitensis* did not appear for 4 days, whereas the saprophytic organism grew vigorously in 48 hours. Accordingly, carbolised media were abandoned during the research.

Exposure to a temperature of 42° C., and the presence of malachite green, carbolic acid and sodium taurocholate, having failed to restrain the growth of bacteria present in urine obtained from Malta fever

patients after careful sterilisation of the external parts, growth under anaërobic conditions was tried but with equally unsatisfactory results. It now appeared evident that in the study of urine all restraining influences must be abandoned and efforts made to obtain as free a growth of the microbes as possible, trusting to subsequent dilution to obtain isolated colonies for purposes of study. Experimentally, this procedure succeeded well enough when the *Micrococcus* was added in considerable quantity to urine, but when the amount inoculated was small, isolation of the *Micrococcus* could not be effected. Trials were then made as to the effect of adding a strong specific serum to these latter growths; it was thought that the serum might cause the aggregation of the *Micrococci* into clumps, and if these were planted out on agar plates a better chance of success might be obtained. The results were encouraging, and in future examinations of the urine of Malta fever cases, it is intended to follow this procedure, as well as the usual dilution method on agar plates.

Experiment I.

An investigation was now undertaken to ascertain whether the *M. melitensis* could live in urine, and especially in a urine which had become alkaline from the decomposition of urea.

A freshly passed urine from a healthy man was inoculated with an emulsion of *M. melitensis* made in distilled water from a recent agar slope. The urine when passed appeared practically sterile. The inoculated urine was placed in a laboratory cupboard and examined daily by plating on nutrose agar. The *Micrococcus* was easily recovered up to and on the 6th day, but could not be detected at a later period. The urine on the 6th day was markedly alkaline from the presence of ammonia, and on titrating it with N/10 acid, the ammonia was found to equal 0.0064 gramme NH_3 per c.c.

This result is of some practical importance as it shows that the *M. melitensis* might be recovered from a urine which had been kept for 6 days and become alkaline in reaction.

The viability of the *M. melitensis* in the presence of ammonia and the comparative absence of saprophytic microbes from the urine in the experiment just related, suggested that, perhaps, this alkali might have a restraining influence on the growth of the bacteria usually found in the urine of Mediterranean fever cases, and so assist in the separation of the specific microbe. Accordingly, broth (+ 10) was treated with pure NH_3 until the amount when titrated with N/10 acid equalled 0.64 gramme per litre. The tubes were incubated and remaining sterile, were then inoculated with *M. melitensis* and with urine from a case of Mediterranean fever. After 24 hours' incubation there was a marked growth of bacteria in the tubes inoculated with urine, but the *M. melitensis* did not show any marked growth until the 4th day. The

result was not unexpected as the work previously done on the reaction of media had shown that the *M. melitensis* did not grow well in alkaline media.

Experiment II.

This experiment was designed in order to ascertain the duration of life of *M. melitensis* when maintained in an absolutely dry state and without a trace of nutrient medium.

A series of sterile cover glasses were placed in a Petri dish and then inoculated with an equal quantity of an emulsion of *M. melitensis*, the cocci from a 48 hours' agar slope being suspended in water. The emulsion was exposed to the air until all traces of moisture had disappeared from the cover glasses. The Petri dish was then placed in a laboratory cupboard, the temperature of which averaged 18° C. Every 24 hours a cover slip was removed and planted out in broth. The resulting growth was plated on agar, and the colonies fished and examined in the following manner:—A likely colony was made into an emulsion with a loopful of broth and then examined under $\frac{1}{2}$ th objective; if cocci were found freely disseminated through the field and showing no signs of clumping, a loopful of serum from an inoculated rabbit was added. When clumping occurred the needle, which had been used to make the emulsion and *not* sterilised, was rubbed over an agar slope. The resulting growth was planted out in glucose peptone, lactose peptone, cane sugar peptone, litmus milk, peptone and salt solution, nitrate broth, and stabbed into gelatine. The growths which resulted corresponded exactly to those obtained when the original *M. melitensis* was planted out in these media.

Result.—A Micrococcus, which corresponded in every particular to the *M. melitensis*, was isolated up to and on the 16th day.

Experiment III.

The object of this experiment was to ascertain the duration of the life of *M. melitensis* in dry soil.

Some soil from a recently manured plot of ground in Gibraltar was powdered, dried, and sterilised, and then inoculated with an aqueous emulsion of *M. melitensis* prepared from an agar slope. The soil was allowed to dry naturally and kept in the laboratory cupboard mentioned in the previous experiment. For a few days traces of moisture were present, but after the 10th day the soil was quite light and formed a black powder which could easily be blown about. The soil was tested weekly for the presence of *M. melitensis*, a portion of the soil being planted out in broth and the resulting growth treated in the manner detailed under Experiment II. Up to and on the 69th day a Micrococcus was recovered, corresponding in every way to the *M. melitensis* originally planted out. During this experiment

careful watch was kept for any change in the morphology of the inoculated microbe. It was thought that the bacillary forms described by Durham might appear, and cause some difficulty in diagnosing the culture. The bacillary forms were never seen, and the Micrococcus obtained on an agar slope on the 69th day presented the usual morphology. The cultural characteristics and reaction to the specific serum were also unchanged.

Result.—The *M. melitensis* retained its vitality in dry soil for 69 days.

Experiment IV.

In this experiment a fine sterile sand, practically free from organic matter, was inoculated, and treated in exactly the same manner as the manured soil in Experiment III. The *M. melitensis* was recovered up to and on the 20th day, but not later. The morphology, cultural and serum reactions, were again quite unchanged.

Result.—The *M. melitensis* retained its vitality in dry sand for 20 days.

Experiment V.

The object of this experiment was to discover the duration of life in a foul soil saturated with water. The manured sterile soil employed in Experiment III was inoculated in the same manner as before, but instead of being allowed to dry it was kept saturated with sterile tap-water. The *M. melitensis* was recovered up to and on the 7th day, but could not be detected at a later date, although many trials were made. The result of this experiment seemed to show that immersion in water was inimical to the persistence of the *M. melitensis* in a saprophytic condition.

Result.—The *M. melitensis* retained its vitality in a foul, saturated soil for 7 days.

Experiment VI.

The idea of this experiment was to ascertain the duration of life of the *M. melitensis* when dried on fabrics. Accordingly, pieces of thick regulation blanket, khaki serge, and khaki cotton were inoculated with an emulsion of the microbe made by suspending a recent agar growth in sterile water. The greatest care was taken not to remove any of the nutrient medium. After inoculation the infected fabrics were placed in a Petri dish and allowed to dry naturally; they were then placed in the laboratory cupboard during the whole experiment. Portions of the fabrics were planted out in broth every 3 or 4 days, and the resulting growth plated on nutrose agar in the usual manner. The *M. melitensis* was recovered from the khaki cotton up to and on the 80th day, from the khaki serge on the 80th day, and from the blanket on the 80th day. The morphology, cultural and serum reactions, were again quite unchanged.

Experiment VII.

The rapid disappearance of the *M. melitensis* from the soil saturated with water suggested that an attempt should be made to determine the duration of life of the *M. melitensis* in sterile water. The whole of a recent growth from an agar slope was diffused in 50 c.c. of sterile tap-water, representing an exceedingly gross pollution. The flask was kept in the laboratory cupboard, and every day 1 c.c. was plated on nutrose agar. The Micrococcus was readily isolated for 6 days, but on the 7th and 13th days it could not be detected.

Experiment VIII.

This experiment was a repetition of Experiment VII, but instead of planting out small quantities from day to day, the flask was left undisturbed for 3 weeks. Broth was then added so as to enrich the whole bulk of the water, and the flask incubated at 37° C. for 3 days. The growth which resulted was found to contain numerous small cocci decolorised by Gram's method. A portion of the growth was then added to an equal quantity of a strong rabbit serum diluted 1—10, and the whole thoroughly mixed was drawn up into a capillary pipette. Distinct agglutination having occurred, the pipette was then opened and the agglutinated mass stroked over a series of agar plates; unfortunately a pure culture of the *M. melitensis* was not obtained. The result of this experiment is not conclusive, but it suggests that the duration of life of the *M. melitensis* in water may be longer than 1 week.

Conclusions.

- (1) The *M. melitensis* is able to live for 6 days in a urine which has become alkaline from the presence of ammonia.
- (2) The *M. melitensis* survives for 16 days when spread in a thin layer on a glass cover slip.
- (3) The *M. melitensis* survives for 69 days when planted in a dry sterilised manured soil.
- (4) In dry sterilised sand the duration of life of the *M. melitensis* appears to be only 20 days.
- (5) In a sterilised manured soil saturated with water the *M. melitensis* appears to survive for only 7 days.
- (6) The *M. melitensis* is able to live for 80 days on dry fabrics, such as blanket, khaki serge, and khaki cotton.
- (7) The *M. melitensis* appears to live for a comparatively short time in sterilised tap-water. It was only recovered in pure culture 6 days after being planted out, though from the result of Experiment VIII it appears possible that the duration of life may extend to 3 weeks.

2.

FURTHER STUDIES ON THE SAPROPHYTIC EXISTENCE OF *MICROCOCCUS MELITENSIS*.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

1. DURATION OF LIFE OF THE *M. melitensis* IN STERILISED TAP- WATER.

Experiment I.

In the Gibraltar report it was stated that the duration of life of the *M. melitensis* in sterilised tap-water was probably longer than the recorded experiments indicated. Accordingly, the experiment of adding an emulsion of *M. melitensis*, made by carefully mixing the growth from an agar slope in sterile water to a known volume of water, was repeated. In this case 1 c.c. of an emulsion made from a strain of *M. melitensis* isolated from urine was added to 10 c.c. of sterilised tap-water. Chemical analysis showed that the tap-water was very pure, and contained practically no organic material. The emulsion was added to the tap-water on August 1, 1904, and at various times 0.5 c.c. was removed, and added to 10 c.c. of broth, the contents of the tube being thoroughly mixed and then incubated at 37° C. As soon as the broth tube showed any signs of growth a large loopful was stroked in a zig-zag manner over an agar slope, which was then incubated at 37° C. On August 15, 1904, a pure culture of *M. melitensis* was isolated, the growth responded to all the usual cultural tests, and agglutinated at once with the serum of Monkey 45, diluted 1—1000. On August 21, 1904, the same procedure was followed, and the *M. melitensis* again isolated. On August 27, 1904, a pure culture of *M. melitensis* was obtained, and appeared quite unchanged. On September 6, 1904, the specific microbe was again isolated.

Result.—The *M. melitensis*, derived from urine, appears to survive for 37 days in sterilised tap-water.

2. DURATION OF LIFE OF THE *M. melitensis* WHEN PLANTED OUT IN SOIL.

In the Gibraltar experiments already recorded a manured garden soil and a dry sand were employed. Valletta and Sliema are mainly built on the Globigerina limestone, and the white dust which abounds on the roads is chiefly due to the attrition of this stone; occasionally the soil has a red colour, due to the presence of oxide of iron resulting from the oxidation of FeS_2 (iron pyrites).

Experiment II.

A grey coloured soil was obtained from Sliema, and ground into a fine powder. According to Sir John Murray's analysis, this soil has the following composition:—

| | |
|--|-------------|
| Carbonate of lime, iron, and alumina ($\text{CaCO}_3, \text{Fe}_2\text{O}_3, \text{Al}_2\text{O}_3$) | 78.39 |
| Phosphate of lime ($\text{Ca}_3\text{2PO}_4$) | 2.70 |
| Magnesium carbonate (MgCO_3) | 0.44 |
| Calcium sulphate (CaSO_4) | 0.33 |
| Insoluble in dilute HCl (1—10) | 17.87 |
| | <hr/> 99.73 |

The soil was carefully dried and sterilised, and a portion planted out in broth and incubated at 37°C . After 4 days' incubation there were no signs of growth, showing that sterilisation had been effected. On July 15, 1904, the soil was inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope in distilled water, and allowed to dry naturally. On July 23, 1904, a portion of the soil, still showing faint traces of moisture, was planted out in broth and incubated at 37°C . On July 26, 1904, a growth occurred in the broth culture, which was planted out on an agar slope; two days later a typical growth, which responded to all the characteristic tests, appeared. On July 30, 1904, the soil was noted to be practically dry. On August 11, 1904, a portion of the soil was removed and treated in the same manner as on July 23, 1904; a typical growth of the *M. melitensis* was again obtained. On August 19, 1904, the same procedure was followed, and a pure culture of the specific microbe was isolated. On August 27, 1904, the *M. melitensis* was again isolated.

Result.—The *M. melitensis* survived for 43 days in a soil, which was allowed to dry naturally, and which was free from appreciable traces of moisture for 27 days.

Experiment III.

In this experiment a reddish coloured soil, also obtained from Sliema, was employed. Sir John Murray's analysis of this soil gave the following results:—

| | |
|---|-------|
| Carbonate of lime (CaCO_3)..... | 80·24 |
| Phosphate of lime ($\text{Ca}_3\text{2PO}_4$)..... | 3·57 |
| Magnesium carbonate (MgCO_3)..... | 1·63 |
| Calcium sulphate (CaSO_4) | 0·06 |
| Iron and alumina (Fe_2O_3 and Al_2O_3)..... | 1·13 |
| Insoluble in dilute HCl (1 in 10) | 12·88 |
| | <hr/> |
| | 99·51 |

The soil was sterilised, and its sterility tested as in Experiment I. On June 25, 1904, it was inoculated with an emulsion of *M. melitensis*, made in sterile water from an agar slope grown for 48 hours at 37° C. The soil, having been dried in the incubator at 37° C., was placed in the laboratory cupboard. On July 4, 1904, a portion of the soil was planted out in broth, and the growth which resulted on July 7, 1904, was planted out on an agar slope. A typical culture, giving all the reactions of the *M. melitensis*, was obtained.

On July 11, 1904, the soil was again tested, and a pure culture of *M. melitensis* was isolated.

On July 15, 1904, an examination was made, but the growth in broth did not take place for 9 days, showing that the organism was much enfeebled. On planting out the growth on agar only a few colonies of the *M. melitensis* were obtained. On July 24, 1904, and on July 30, 1904, examinations were made, but the results were negative, the *M. melitensis* having apparently died out.

Result.—The *M. melitensis* lived for 21 days in red Sliema soil, thoroughly dried immediately after inoculation.

Experiments IV and V.

These experiments were designed in order to ascertain whether the presence of traces of moisture, as distinguished from flooding of the soil, had any influence on the survival of the *M. melitensis*.

In Experiment IV white Globigerina limestone dust was inoculated with *M. melitensis* on July 8, 1904; the tube was then placed in the laboratory cupboard. About once a week a little sterile tap-water was added by means of a pipette, so as to preserve a faint appearance of moisture on the surface of the soil. At various intervals portions of the soil were removed and planted out in broth, the tube being then incubated at 37° C. The resulting growth was planted on agar and tested as already described under Experiment I.

The *Micrococcus melitensis* was isolated on July 15, 1904.

| | | | |
|---|---|---|------------------|
| " | " | " | July 24, 1904. |
| " | " | " | July 30, 1904. |
| " | " | " | August 11, 1904. |
| " | " | " | August 19, 1904. |

The Micrococcus melitensis was isolated on August 27, 1904.

“ “ “ September 7, 1904.

“ “ “ September 19, 1904.

Result.—The *M. melitensis* survived for 72 days in a damp soil.

In Experiment V the red soil, described under Experiment II, was employed. The soil was inoculated on July 8, 1904, and the testings carried out at the same time as in Experiment III. The *M. melitensis* was isolated after 72 days' immersion in this soil.

3. SURVIVAL OF THE *M. melitensis* AFTER EXPOSURE TO THE SUN.

Experiment VI.—Exposure on Thin Strips of Glass.

A 36-hours' growth of *M. melitensis* on nutrose agar was made into an emulsion with sterile tap-water. A series of thin glass cover slips were sterilised and the surface of each inoculated with the emulsion by means of a sterile pipette. The cover slips were then exposed to the sun as follows :—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each exposure one of the cover slips was added to sterile broth and incubated at 37° C. The broth tubes all remained sterile, though the incubation was maintained for 14 days.

From control slips, not exposed to the sun, the *M. melitensis* was easily recovered.

Experiment VII.—Exposure in a Very Thin Layer of Soil.

Samples of white and red soils, already mentioned under the soil experiments, were spread in layers, $\frac{1}{8}$ inch deep, on the bottom of glass dishes, and then inoculated with an emulsion of *M. melitensis*, made from an agar slope as mentioned above. The dishes were exposed to the sun as follows :—

On June 20, 1904, from 12.15 P.M. to 1 P.M. Maximum temperature in the sun, 128° F. (53°·3 C.).

On June 21, 1904, from 8.50 A.M. to 11.50 A.M. Maximum temperature in the sun, 135° F. (57°·2 C.).

On June 22, 1904, from 8.45 A.M. to 11.45 A.M. Maximum temperature in the sun, 126° F. (52°·2 C.).

On July 1, 1904, from 10.30 A.M. to 12.30 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each experiment particles from the dried baked surface were planted out in broth, and any resulting growth was then planted out on agar and the growth tested for agglutination, etc. The *M. melitensis* was recovered after the exposure on June 21, 1904, representing $3\frac{3}{4}$ hours' exposure to direct sunlight, but not later.

The *M. melitensis* was readily obtained from a control soil after 21 days in the laboratory cupboard.

Experiment VIII.—Exposure on Khaki Drill.

A piece of khaki drill was inoculated with the same emulsion used in the previous experiments. The drill was then exposed to the sun as follows:—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. ($54^{\circ}\cdot4$ C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun 130° F. ($54^{\circ}\cdot4$ C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. ($56^{\circ}\cdot1$ C.).

After each exposure a portion of the infected drill was cut off and planted out in broth, and the resulting growth planted out on agar and tested in the usual manner.

The *M. melitensis* was recovered after an exposure of not more than $2\frac{1}{2}$ hours to the sun.

Experiment IX.—Exposure on Soil $\frac{1}{2}$ -inch Deep.

The idea of this experiment was to ascertain whether the deeper layers of the soil, which were quite dry and capable of being blown about by strong winds, would still retain infection after exposure to the sun.

The white Globigerina limestone soil, previously described, was sterilised and carefully poured into a sterile Petri dish so as to form a uniform layer $\frac{1}{2}$ inch deep. The soil was then inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope, inoculated from a urine culture and incubated for 48 hours at 37° C. The soil was exposed to the sun as follows:—

August 19, 1904, 3.30 P.M. to 4.30 P.M. Maximum temperature in the sun, 147° F. ($63^{\circ}\cdot8$ C.).

August 20, 1904, 9 A.M. to 11.45 A.M. Maximum temperature in the sun, 153° F. ($67^{\circ}\cdot2$ C.). After the total exposure of $3\frac{3}{4}$ hours, a portion from the surface was planted out in broth, so as to compare this experiment with the one previously reported.

August 21, 1904, exposed from 9.30 A.M. to 11.30 A.M. Maximum temperature in sun, 154° F. ($67^{\circ}\cdot7$ C.). After a total exposure of

5½ hours, portions of soil taken from the surface and from the depth were planted out in broth tubes.

August 22, 1904, exposed from 9 A.M. to 11.15 A.M. Maximum temperature in sun 148° F. (64°·4 C.). Portions of soil from the surface and depth again planted out in broth.

August 23, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 148° F. (64°·4 C.). Planted out portions of soil from the surface and depth in broth tubes.

August 25, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 146° F. (63°·3 C.). Total exposure since the 19th equals 10 hours. Planted out portions of soil from the surface and depth in broth tubes.

September 6, 1904. All the broth tubes which had been incubated at 37° C., since the date of inoculation, were planted out on agar slopes.

September 12, 1904. All the agar tubes inoculated with the broth containing the surface soil, have remained quite sterile.

September 12, 1904. The agar tubes inoculated with the broth containing the portions of soil taken from the depth after 5½ and 8 hours' exposure, show a growth of *B. mesentericus*. There is no sign of the *M. melitensis*.

The agar tubes inoculated with the broth tubes containing the soil from the depth after 9 and 10 hours' exposure are quite sterile.

Result.—The heat derived from exposure to the sun, the maximum temperature varying between 146° F. and 153° F., apparently destroys the *M. melitensis* at a depth of ½ inch from the surface.

Experiment X.—Duration of Life of the M. melitensis when Planted out in Sea-Water.

Sea-water was obtained from the harbour and sterilised. A portion was then planted out on agar and in broth; both the tubes remained sterile after incubation at 37° C.

On July 25, 1904, a tube containing 10 c.c. of sterile sea-water was inoculated with the growth obtained from an agar slope, incubated for 13 days at 37° C. The inoculated tube was placed in the laboratory cupboard. On July 29, 1904, 0·5 c.c. was removed from the tube and planted out in broth; on September 2, 1904, there was a distinct growth in the broth; the growth was planted out on an agar slope, and a typical growth of *M. melitensis* was obtained, which responded to the classical tests.

On July 31, 1904, 0·5 c.c. was planted out in broth, and the same procedure followed as on July 29, 1904; a typical growth of *M. melitensis* was obtained.

On August 5, 1904, 0·5 c.c. was planted out in broth; a growth of *M. melitensis* resulted.

On August 8, 1904, 0.5 c.c. was planted out as before, and a pure culture of *M. melitensis* was obtained.

On August 12, 1904, 0.5 c.c. was planted out in broth; the resulting growth when planted on an agar slope gave rise to a growth, which agglutinated very slowly with the serum from Monkey 45. A portion of the growth was planted out in glucose and litmus milk; the glucose was not fermented, and the litmus milk became alkaline, without showing the slightest trace of coagulation or digestion. The growth also had a typical morphology, and did not stain by Gram's method.

On August 15, 1904, 0.5 c.c. was planted out in broth, and a culture again obtained, which was typical of *M. melitensis*, except that the agglutination occurred slowly.

On August 19, 1904, 0.5 c.c. was planted out, and the same result obtained as on August 12 and 15, 1904. The growth was tested with the specific serum which, diluted 1—1000, caused instantaneous agglutination of the laboratory standard culture of *M. melitensis*. With the growth from sea-water, this serum, diluted 1—1000, caused clumping in $\frac{1}{2}$ hour.

On August 22, 1904, 0.5 c.c. was planted out in broth, and incubated at 37° C. No sign of growth appeared after 15 days' incubation.

On August 26, 1904, 0.5 c.c. was again planted out, but no growth appeared.

Result.—The *M. melitensis* appears to survive for 25 days in sterilised sea-water.

Conclusions.—1. The *M. melitensis* retains its vitality in sterilised tap-water for 37 days.

2. In a Maltese soil, allowed to dry naturally, the *M. melitensis* survives for 43 days; and in one thoroughly dried immediately after inoculation, it survives for 21 days.

3. The *M. melitensis* survives for 72 days in a damp soil.

4. Exposure to the sun for a few hours kills the *M. melitensis*.

5. The *M. melitensis* survives for 25 days in sterilised sea-water.

3.

ON THE RECOVERY OF THE *MICROCOCCUS MELITENSIS* FROM THE URINE, FÆCES, AND SWEAT OF PATIENTS SUFFERING FROM MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

Note.—The work on the examination of urine, fæces and mosquitoes has been done
in conjunction with Captain Kennedy, R.A.M.C.

1. *Examination of Urine.*

In my report on previous work performed at Gibraltar, it was pointed out that the ordinary restraining agents, such as carbolic acid, sodium taurocholate, malachite green, etc., could not be depended upon to inhibit the growth of the micro-organisms usually found associated with the *M. melitensis* in the urine of Mediterranean fever cases. Accordingly, in the earlier work at Malta, attempts were made to isolate the Micrococcus by first enriching a known bulk of urine with broth, usually in the proportions of 1—1 and 1—3, and then, after varying periods of incubation at 37° C., plating the growths, which resulted, on nutrose agar. It was hoped that, under these conditions, the specific microbe would so multiply as to enable colonies to be detected by the plate method. A very short experience showed that the enrichment method was not satisfactory; the extraneous organisms multiplied more vigorously than the *M. melitensis*, and the latter was completely crowded out. It was then decided to make use of the glucose-litmus-nutrose-agar plates, already mentioned in the Gibraltar report, and to add small quantities of urine, 0·25—0·33 c.c., to these plates, allowing the urine to flow over and form a thin layer on the surface of the solidified agar. This procedure enabled the actual number of colonies of the Micrococcus passed in the urine to be ascertained. Before collecting the urine for investigation, the genitalia were washed with carbolic acid lotion; the patient then passed urine, but the first portion, which acted as a flush to the urethra, was discarded. On the glucose-litmus-nutrose-agar plates, the colonies of the *M. melitensis* appeared as almost transparent deep blue drops; likely colonies were next fished, and made into an

emulsion with normal salt solution on a cover-glass. It may be noted that the *M. melitensis* readily emulsifies, and the culture appears to flow off the point of the needle into the surrounding fluid; this characteristic was found of great assistance in detecting the specific microbe. A streptococcus is found in urine which produces, on the special plates, colonies very closely resembling those of the *M. melitensis*; when fished, however, they do not readily emulsify, and, on examination, under one-twelfth, are found to consist of a medium-sized coccus, staining with Gram. When it was found that the colony readily emulsified, the hanging drop was carefully examined under the oil immersion, in order to ascertain the nature of the organism, and to make sure that no false clumps were present. If the microbe presented the characteristics of the *M. melitensis*, and the emulsion was satisfactory, the cover-glass was removed, and a little specific animal serum added. In the earlier work I employed a rabbit serum prepared at Gibraltar, but, in the later work, serum from Monkey 45 was used. When the microbe under examination manifested instantaneous clumping under the influence of the serum, a portion of the colony was planted out on an agar slope, and incubated at 37° C. The resulting growth was then treated as follows:—

(1) Tested with the serum of Monkey 45. This serum, when diluted 1—1000, was found to cause instantaneous clumping, visible to the naked eye, of the laboratory stock culture of *M. melitensis*.

(2) Planted in glucose-litmus-peptone, or on a glucose-litmus-agar slope, and incubated for 7 days at 37° C.

(3) Planted in litmus milk and incubated for a month at 37° C.

(4) Examined as to retention of stain by Gram's method.

A micro-organism, which agglutinates with a specific animal serum in a high dilution, does not ferment glucose, renders milk alkaline without coagulation, may justly be regarded as the *M. melitensis*.

All the strains of *M. melitensis*, which have been isolated from the urine of Mediterranean fever cases, have responded to these tests.

Employing the above technique the first successful isolation was obtained from the urine of Sergeant Pudney, 2nd Essex Regiment. A plate made with 0.33 c.c. of urine was found to contain thirty-three colonies, after 5 days' incubation at 37° C.; colonies were first observed on the 4th day, but the maximum number did not appear until the 5th day of incubation.

The *M. melitensis* has now been isolated thirty-nine times, and from the urine of thirteen different patients. Colonies were never observed before the 3rd day of incubation, and at this period they were usually very minute and easily missed; on the 4th day of incubation, however, they were readily detected on the glucose-litmus-nutrose-agar plates. The actual numbers of *M. melitensis* isolated from urine are shown in the attached table (A).

Table A.—Showing the Number of Colonies of *M. melitensis* found in each Sample of Urine.

| Name. | Date. | Quantity of urine (in c.c.) | No. of colonies in each plate. | No. of colonies per c.c. | Average No. per c.c. |
|----------------|---------|---|--------------------------------|--------------------------|----------------------|
| Howe | 6.7.04 | Isolated from broth culture made with urine obtained at the <i>post-mortem</i> examination. | | | |
| Pudney | 18.7.04 | 0·5 | 1 | 2 | 50 |
| | | 0·33 | 33 | 99 | |
| „ | 25.7.04 | 0·25 | 3 | 12 | 26 |
| | | 0·25 | 3 | | |
| | | 0·25 | 3 | | |
| | | 0·25 | 3 | | |
| | | 0·25 | 21 | 84 | 6 |
| | | (mucus) | | | |
| „ | 27.7.04 | 0·33 | 2 | 6 | 6 |
| Martin | 2.8.04 | 0·33 | 95 | 285 | 285 |
| Markham | 6.8.04 | 0·25 | 5 | 20 | 20 |
| Breuster | 6.8.04 | 0·25 | 2 | 8 | 8 |
| Belfield | 6.8.04 | 0·33 | 1 | 3 | 3 |
| Pudney | 7.8.04 | 0·125 | 1 | 8 | 8 |
| „ | 8.8.04 | 0·5 | 4 | 8 | 8 |
| Fisher | 8.8.04 | 0·33 | 3 | 9 | 15 |
| | | 0·33 | 7 | 21 | |
| Lawson | 12.8.04 | 0·25 | 1 | 4 | 4 |
| Breuster | 13.8.04 | 0·25 | 2 | 8 | 8 |
| Lawson | 14.8.04 | 0·33 | 4 | 12 | 12 |
| „ | 14.8.04 | 0·33 | 5 | 15 | 15 |
| „ | 15.8.04 | 0·25 | 6 | 24 | 24 |
| Lawrence | 16.8.04 | 0·25 | 1 | 4 | 4 |
| | | 0·25 | 1 | 4 | |
| Lawson | 17.8.04 | 0·25 | 45 | 180 | 180 |
| Fisher | 19.8.04 | 0·25 | 1 | 4 | 4 |
| Lawson | 20.8.04 | 0·33 | 12 | 36 | 36 |
| Griffin | 20.8.04 | 0·25 | 10 | 40 | 40 |
| Lawson | 21.8.04 | 0·33 | 15 | 45 | 45 |
| Griffin | 23.8.04 | 0·25 | 1 | 4 | 4 |
| Lawson | 23.8.04 | 0·33 | 105 | 315 | 315 |
| Pudney | 23.8.04 | 0·33 | 2 | 6 | 6 |
| Breuster | 24.8.04 | 0·25 | 1 | 4 | 4 |
| Griffin | 24.8.04 | 0·25 | 1 | 4 | 4 |
| Lawson | 26.8.04 | 0·33 | 1 | 3 | 3 |
| Markham | 26.8.04 | 0·33 | 1 | 3 | 3 |
| Lawson | 29.8.04 | 0·33 | 3 | 9 | 9 |
| Barry | 29.8.04 | 0·33 | 1 | 3 | 3 |
| Christie | 29.8.04 | 0·33 | 4 | 12 | 12 |
| Lawson | 31.8.04 | 0·33 | 1 | 3 | 3 |
| Lawrence | 1.9.04 | 0·33 | 3 | 9 | 9 |
| Markham | 1.9.04 | 0·33 | 1 | 3 | 3 |
| Griffin | 9.9.04 | 0·33 | 5 | 15 | 15 |
| Lawrence | 10.9.04 | 0·33 | 70 | 210 | 210 |
| Kinsella | 16.9.04 | 0·33 | 1 | 3 | 15 |
| | | 0·33 | 9 | 27 | |
| Markham | 28.9.04 | 0·5 | 298 | 596 | 596 |

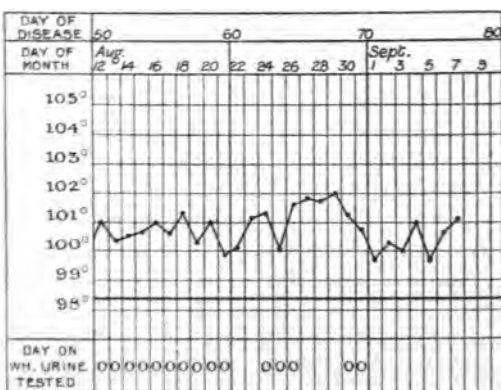
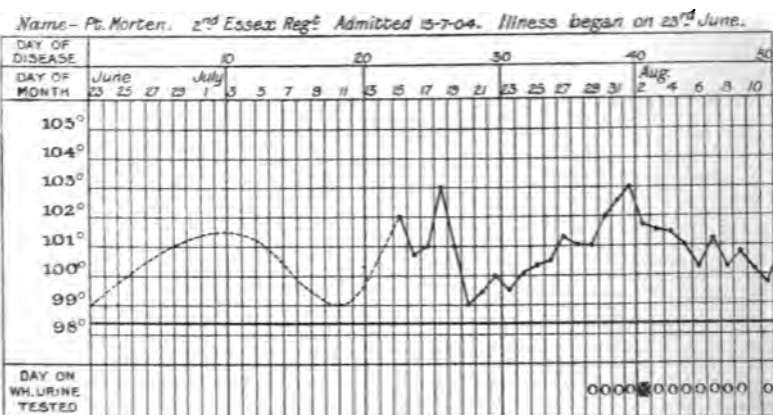
Up to the present time the *Micrococcus* has not been isolated from urine earlier than the 15th day or later than the 82nd day of disease.

It is present in the urine of patients who are sufficiently convalescent to be allowed up, but still have an evening rise of temperature.

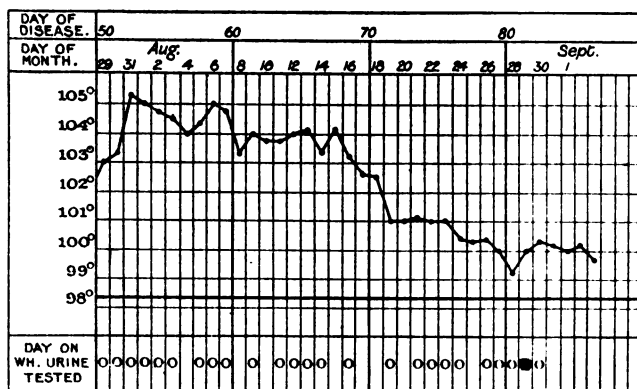
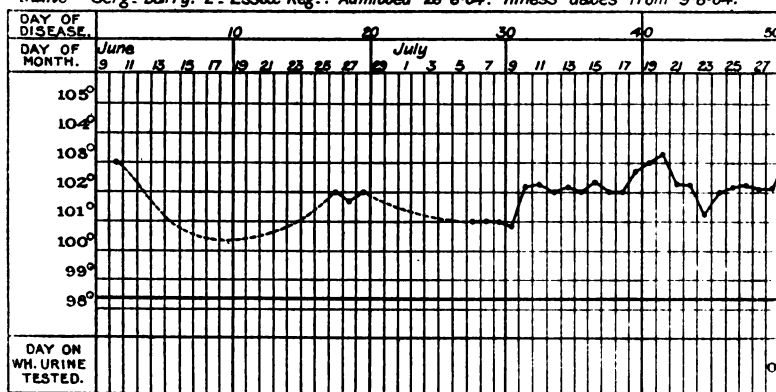
In order to save repetition and to enable the work done to be grasped at a glance, the attached charts have been prepared by Captain Kennedy, Royal Army Medical Corps, who has given me most valuable assistance throughout the work. Each square represents a day of disease, and in every case the chart commences with the day which, after careful questioning of the patient, was considered to be probably the 1st day of disease; so that on looking through the charts the different columns represent the same day of disease for each patient. The course of the fever is represented by the evening temperature, and the 0 sign indicates an examination made without any result; the Maltese cross sign represents a successful isolation of the *M. melitensis*. It will be noticed that there are many failures as compared with successes. In the earlier work the constant want of success was undoubtedly due to the faulty method of procedure; but in the later work it is to be attributed partly to the fact that the *M. melitensis* is not voided in the urine every day, but appears in gushes at uncertain periods, and partly to the presence in the urine of acid-producing organisms, which out-grow and interfere with the development of the *M. melitensis*.

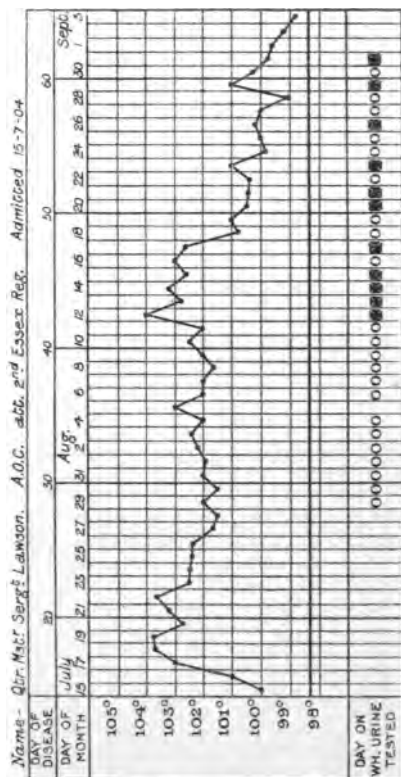
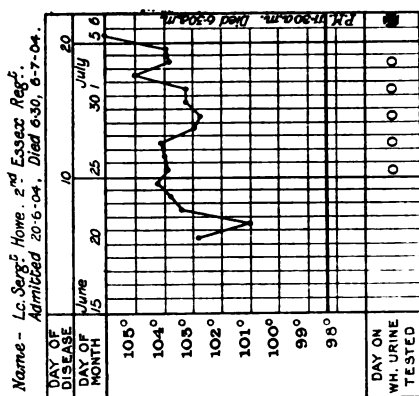
Careful observation of the urines has been made in order to ascertain whether any physical or chemical change is associated with the passage of the *M. melitensis*. All the urines have been free from the general opacity or turbidity, which is associated with Typhoid Bacilluria. A little deposit of mucus has been observed, and a portion of this when plated out has always given more colonies than the clear portion of the urine treated in the same manner. On three occasions a trace of albumen was noticed, but up to the present no physical or chemical change common to all the urines and indicating the passage of the *M. melitensis* has been observed.

Table A shows the number of micrococci per cubic centimetre obtained from each sample of urine, and indicates the dates when the isolation was effected. It will be noticed that the numbers of micrococci excreted are small as compared with the figures recorded by several observers during the bacilluria of typhoid fever. It is possible that the figures given in the table do not represent the actual numbers passed in every case, and that many colonies escaped observation owing to their being crowded out by other microbes. At the same time many of the plates, notably those of Sergeant Pudney and Private Lawson, were nearly pure cultures of *M. melitensis*, and as all the colonies which appeared were perfectly discrete, and there was ample room in the plates for other colonies to develop had they been present, it does not seem probable that the numbers passed greatly exceeded the maximum figures recorded.

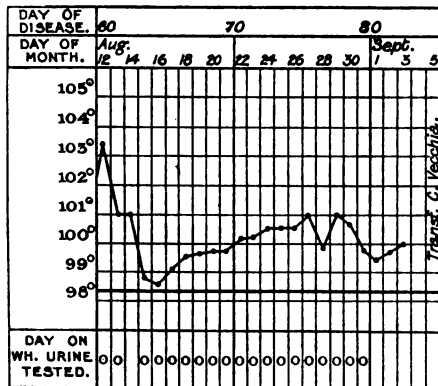
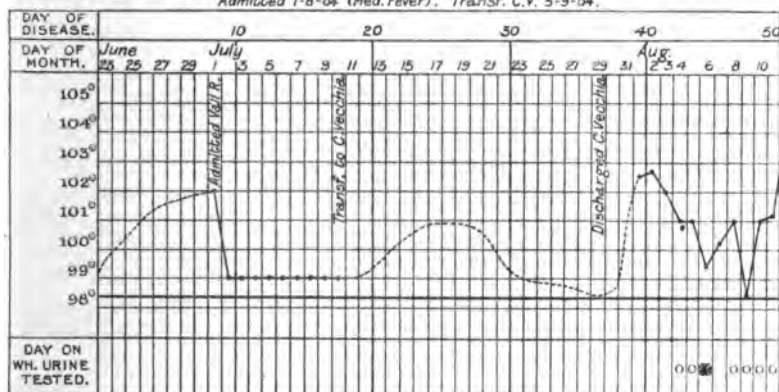


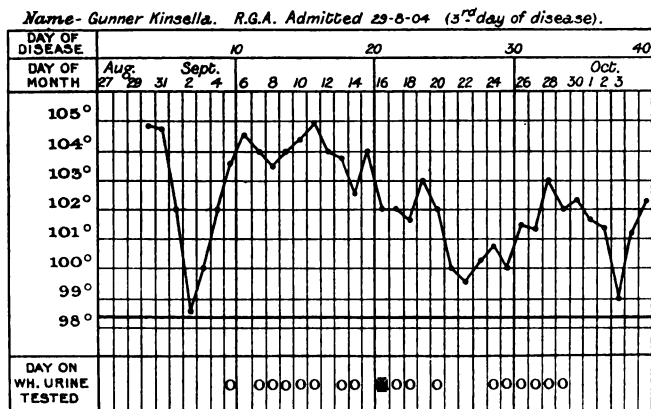
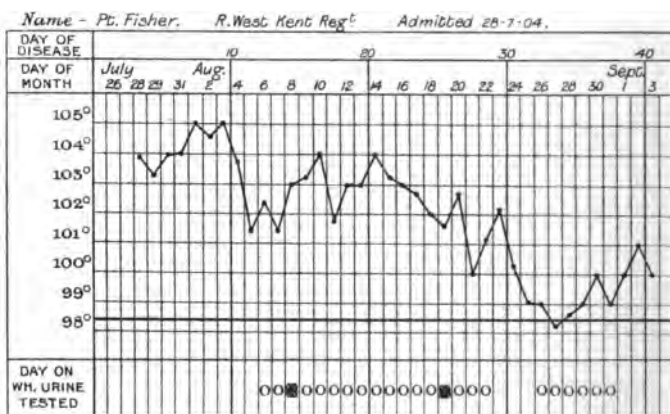
Name - Sergt Barry. 2nd Essex Regt. Admitted 26-6-04. Illness dates from 9-6-04.



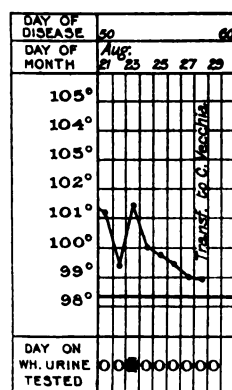
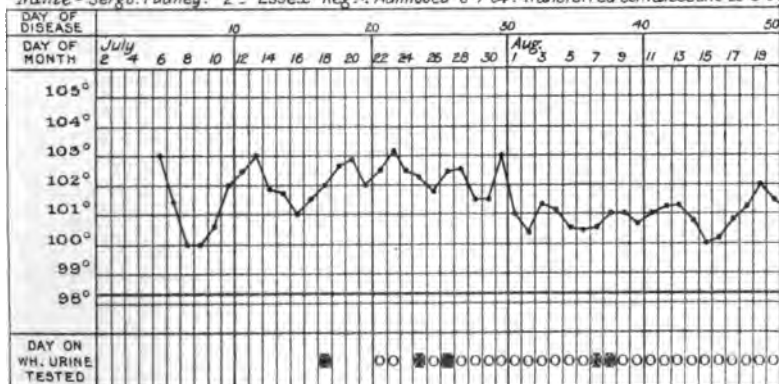


Name - Boy Bellfield. R. West Kent Regt. Admitted S.C.F. (??) 1-7-04. Transf. C.V. 10-7-04.
Admitted 1-8-04 (Med. Fever). Transf. C.V. 5-9-04.

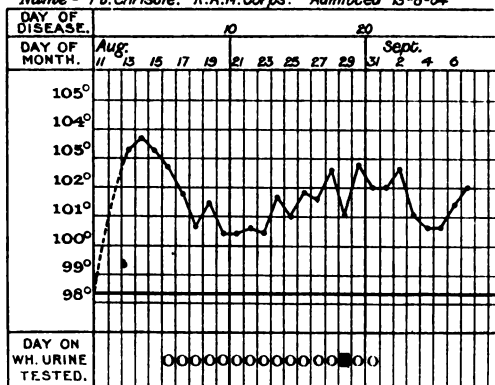


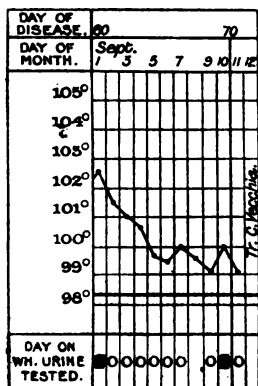
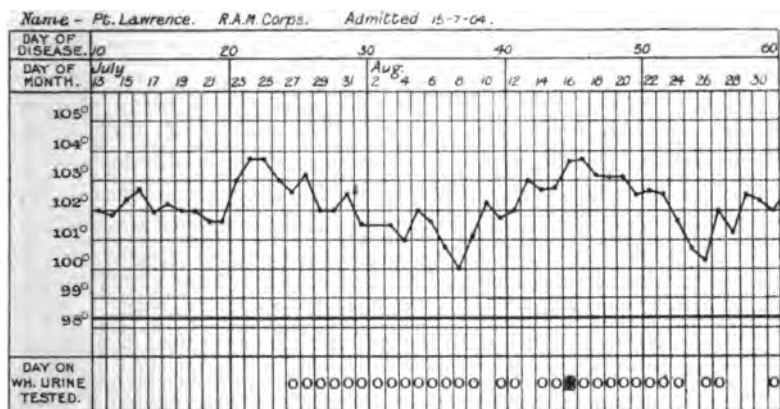
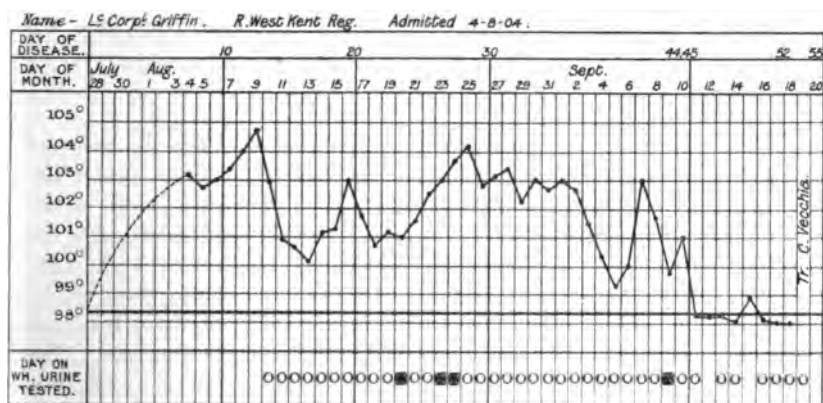


Name - Sergt. Pudney. 2nd Essex Regt. Admitted 6-7-04. Transferred convalescent 29-8-04



Name - Pte. Christie. R.A.M. Corps. Admitted 13-8-04





Up to the present 520 samples of urine have been examined, representing the study of more than 1000 plates.

2. Examination of Fæces.

Having succeeded in isolating the *M. melitensis* from the urine of Mediterranean fever cases, attempts were now made to detect the microbe in the fæces of these patients. Unfortunately, most of the cases suffered from constipation, and the bowels only acted after the administration of an enema. A few patients suffered from diarrhoea for a short time, and the opportunity was taken of investigating these stools.

The great difficulty to contend with in the study of fæces is caused by the presence of the rapidly growing *B. coli* in large numbers. The enrichment method, which failed with the urine, appeared to be even less likely to yield satisfactory results with fæces. A few trials were made of planting out some of the stools in broth and then, after incubating for four days at 37° C., plating out the growths on glucose-litmus-nutrose-agar plates. The results were highly unsatisfactory; the *B. coli* and its allies converted the plates into a strongly acid medium, on which the *M. melitensis* would not grow. Evidently a medium on which the *B. coli* could not develop, would prove of great assistance in isolating the *M. melitensis* from stools. E. Roth in the *Archiv f. Hygiene* of March 3, 1904, reported that the development of the *B. coli* was arrested in a medium containing 60 per cent. of a solution containing $\frac{1}{100}$ th of caffeine. For greater security against the development of *B. coli* he recommended the proportion of caffeine to be increased to 115 per cent. of the $\frac{1}{100}$ th solution. Ficker and Hoffmann in the same number of the *Archiv f. Hygiene* also attested the value of caffeine in arresting the development of *B. coli*; they used 5 grammes of caffeine per litre of fluid. Courmont and Lacomme also wrote on caffeine in bacteriology in the March number of the *Journal of Physiology and Pathology*, 1904. They stated that when caffeine was added to broth to the extent of 1 per cent., the development of *B. coli* was prevented. In view of these statements, experiments were made to test the viability of the *M. melitensis* in caffeinised media. Broth tubes were prepared containing 0.5 per cent., 0.75 per cent., and 1 per cent. of caffeine. Each tube was inoculated with a small loopful of an agar growth, derived from the spleen of Sergeant Howe. The results obtained were as follows:—

- (1). 5.8.04, 0.5 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.
- (2). 5.8.04, 0.75 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.
- (3). 5.8.04, 1 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.

(1). 8.8.04, 0.5 per cent. Good growth. Planted out on agar and *M. melitensis* recovered.

(2). 8.8.04, 0.75 per cent. No growth.

(3). 8.8.04, 1 " "

(2). 9.8.04, 0.75 " "

(3). 9.8.04, 1 " "

(2). 10.8.04, 0.75 " "

(3). 10.8.04, 1 " "

(2). 11.8.04, 0.75 " "

(3). 11.8.04, 1 " "

(2). 12.8.04, 0.75 " "

(3). 12.8.04, 1 " "

(2). 15.8.04, 0.75 " "

(3). 15.8.04, 1 " "

(2). 18.8.04, 0.75 per cent. No growth. Planted out on agar slopes. No growths appeared.

(3). 18.8.04, 1 per cent. No growth. Planted out on agar slopes. No growths appeared.

Result.—*M. melitensis* derived from the spleen of man does not appear to develop in media containing more than 0.5 per cent. of caffeine.

Courmont and Lacomme having stated in their paper that cultures of *B. typhosus* from urine were more resistant to the action of caffeine than cultures derived from the blood, experiments were made to see if the same held good for cultures of *M. melitensis*. Accordingly, batches of the same broth used in the previous experiments were inoculated with an agar culture obtained from Sergeant Pudney's urine; the tubes were incubated at 37° C.

The results obtained were as follows :—

(1). 5.8.04, 0.5 per cent. Caffeine broth, inoculated with culture from urine.

(2). 5.8.04, 0.75 per cent. Caffeine broth, inoculated with culture from urine.

(3). 5.8.04, 1 per cent. Caffeine broth, inoculated with culture from urine.

(1). 8.8.04, 0.5 per cent. Good growth. Planted on agar. *M. melitensis* recovered.

(2). 8.8.04, 0.75 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

(3). 8.8.04, 1 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

Result.—The *M. melitensis* derived from urine is able to grow, but only feebly, in broth containing 0.75 and 1 per cent. of caffeine.

A culture of *B. coli* isolated from the stool of a Mediterranean fever case was next tested as to its growth in caffeinised broth. The results obtained were as follows :—

(1). 16.8.04, 0·5 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(2). 16.8.04, 0·75 per cent. Caffeine broth inoculated with *B. coli* from stool of Mediterranean fever case.

(3). 16.8.04, 1 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(1). 17.8.04, 0·5 per cent. Good growth. Planted on agar. *B. coli* recovered.

(2). 17.8.04, 0·75 per cent. No growth.

(3). 17.8.04, 1 " "

(2). 18.8.04, 0·75 " "

(3). 18.8.04, 1 " "

(2). 19.8.04, 0·75 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

(3). 19.8.04, 1 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

Result.—Caffeine in the proportion of 0·75 and 1 per cent. appeared to have a distinct restraining influence on the growth of *B. coli*.

An emulsion of one loop of *B. coli* and one loop of *M. melitensis*, from a urine culture, was now thoroughly mixed and then plated out on 0·75 per cent. caffeine-glucose-nutrose-litmus-agar. As a result a few colonies of *B. coli* appeared in 48 hours, but no signs of the *M. melitensis* were observed even after 6 days' incubation at 37° C.; evidently the use of media containing more than 0·50 per cent. of caffeine would be attended with considerable risk of arresting the growth of the *M. melitensis*.

A batch of plates, containing 0·5 per cent. of caffeine in addition to the usual glucose-nutrose-litmus-agar, was now prepared. An emulsion of a stool from a Mediterranean fever case was plated out, and as a control the same emulsion in the same quantities was plated on the ordinary glucose-nutrose-litmus-agar. After 48 hours' incubation at 37° C., there was no appreciable difference between the plates, so the use of caffeine was abandoned in this investigation. The technique has consisted in adding loopfuls of the fluid stools, the number of loops depending on the fluidity of each stool, to either sterile salt solution or broth until a slightly opalescent mixture was produced. Loopfuls of the mixture were then stroked concentrically or diffused by means of a "platinum spreader" over the surface of glucose-litmus-nutrose-agar, solidified in Petri dishes. The plates were then placed with the covers downwards in the 37° C. incubator. After 4 and 5 days' incubation the resulting colonies were examined in a hanging drop; if anything like the morphology of *M. melitensis* appeared, the cover-glass was removed, and a loopful of the specific serum, diluted 1—10, added. Many of the streptococci occurring in stools bear a superficial resemblance to the *M. melitensis*; still, as a rule,

the colonies have a faint opacity and sometimes a reddish tinge which enables them to be at once distinguished from the *M. melitensis*. In any case of doubt the addition of the specific serum enabled a diagnosis to be made. The attached table shows the number of stools examined and the results up to the present time. It will be seen that 1026 plates made from eighty-six stools have been studied, but with a negative result.

Examination of Stools of Mediterranean Fever Cases.

| Name. | Dates. | Number of plates. | Day of disease. | Result. |
|--------------------|---------|-------------------|-----------------|------------------------------------|
| 1. Barry | 31.7.04 | 6 | 53 | <i>M. melitensis</i> not isolated. |
| 2. " | 23.8.04 | 12 | 76 | " " |
| 3. " | 24.8.04 | 4 | 77 | " " |
| 4. Eldred | 27.7.04 | 10 | 27 | " " |
| 5. " | 26.7.04 | 4 | 26 | " " |
| 6. Francis | 17.7.04 | 3 | 19 | " " |
| 7. " | 18.7.04 | 3 | 20 | " " |
| 8. Vince | 23.7.04 | 5 | 18 | " " |
| 9. " | 17.8.04 | 9 | 43 | " " |
| 10. " | 24.8.04 | 4 | 50 | " " |
| 11. Moore | 25.7.04 | 5 | 25 | " " |
| 12. Brewster | 5.8.04 | 5 | 42 | " " |
| 13. Jones | 7.8.04 | 4 | 55 | " " |
| 14. " | 8.8.04 | 3 | 56 | " " |
| 15. " | 9.8.04 | 4 | 57 | " " |
| 16. Griffin | 11.8.04 | 4 | 15 | " " |
| 17. " | 15.8.04 | 8 | 19 | " " |
| 18. " | 16.8.04 | 9 | 20 | " " |
| 19. " | 17.8.04 | 4 | 21 | " " |
| 20. " | 19.8.04 | 4 | 23 | " " |
| 21. " | 21.8.04 | 21 | 25 | " " |
| 22. " | 23.8.04 | 4 | 27 | " " |
| 23. Mays | 12.8.04 | 4 | 40 | " " |
| 24. Fisher | 14.8.04 | 8 | 21 | " " |
| 25. " | 15.8.04 | 19 | 22 | " " |
| 26. " | 16.8.04 | 3 | 23 | " " |
| 27. " | 17.8.04 | 6 | 24 | " " |
| 28. " | 18.8.04 | 16 | 25 | " " |
| 29. " | 19.8.04 | 8 | 26 | " " |
| 30. Christie | 2.9.04 | 21 | 23 | " " |
| 31. Lawrence | 2.9.04 | 8 | 62 | " " |
| 32. Hurrell | 23.8.04 | 24 | 23 | " " |
| 33. Fisher | 23.8.04 | 16 | 30 | " " |
| 34. Hurrell | 25.8.04 | 21 | 25 | " " |
| 35. Vince | 25.8.04 | 14 | 51 | " " |
| 36. Hurrell | 26.8.04 | 30 | 26 | " " |
| 37. Curry | 27.8.04 | 11 | 21 | " " |
| 38. Hurrell | 28.8.04 | 15 | 28 | " " |
| 39. Griffin | 26.8.04 | 16 | 33 | " " |
| 40. Christie | 29.8.04 | 14 | 19 | " " |
| 41. Martin | 8.9.04 | 13 | 20 | " " |
| 42. Christie | 8.9.04 | 15 | 29 | " " |
| 43. Fisher | 8.9.04 | 15 | 46 | " " |
| 44. Campbell | 9.9.04 | 22 | 27 | " " |
| 45. Christie | 9.9.04 | 14 | 30 | " " |

Examination of Stools of Mediterranean Fever Cases—*contd.*

| Name. | Dates. | Number of plates. | Day of disease. | Result. |
|-------------------|---------|-------------------|-----------------|------------------------------------|
| 46. Ingram..... | 9.9.04 | 15 | — | <i>M. melitensis</i> not isolated. |
| 47. Groom..... | 10.9.04 | 20 | 25 | " " |
| 48. Fisher..... | 10.9.04 | 20 | 48 | " " |
| 49. Christie..... | 10.9.04 | 18 | 31 | " " |
| 50. Groom..... | 11.9.04 | 12 | 26 | " " |
| 51. Christie..... | 11.9.04 | 11 | 32 | " " |
| 52. Fisher..... | 11.9.04 | 15 | 49 | " " |
| 53. Groom..... | 12.9.04 | 12 | 27 | " " |
| 54. Gane..... | 12.9.04 | 12 | 23 | " " |
| 55. Christie..... | 13.9.04 | 10 | 34 | " " |
| 56. Silcocks..... | 13.9.04 | 10 | 36 | " " |
| 57. Jones..... | 13.9.04 | 11 | 13 | " " |
| 58. Fisher..... | 14.9.04 | 10 | 52 | " " |
| 59. Christie..... | 14.9.04 | 10 | 35 | " " |
| 60. Silcocks..... | 14.9.04 | 12 | 37 | " " |
| 61. "..... | 15.9.04 | 10 | 38 | " " |
| 62. "..... | 16.9.04 | 10 | 39 | " " |
| 63. Silburn..... | 16.9.04 | 10 | 12 | " " |
| 64. Silcocks..... | 17.9.04 | 20 | 40 | " " |
| 65. Hurrell..... | 19.9.04 | 14 | 50 | " " |
| 66. Silcocks..... | 19.9.04 | 14 | 42 | " " |
| 67. Fisher..... | 19.9.04 | 20 | 57 | " " |
| 68. Barry..... | 20.9.04 | 14 | 104 | " " |
| 69. Smith..... | 20.9.04 | 14 | 25 | " " |
| 70. Silburn..... | 20.9.04 | 14 | 16 | " " |
| 71. Jones..... | 21.9.04 | 14 | 21 | " " |
| 72. Martin..... | 21.9.04 | 12 | 33 | " " |
| 73. Iggo..... | 21.9.04 | 12 | 11 | " " |
| 74. Rowlands..... | 22.9.04 | 12 | 59 | " " |
| 75. Smith..... | 22.9.04 | 12 | 27 | " " |
| 76. Rowlands..... | 23.9.04 | 12 | 60 | " " |
| 77. Smith..... | 23.9.04 | 12 | 28 | " " |
| 78. Silcocks..... | 23.9.04 | 12 | 46 | " " |
| 79. Fisher..... | 24.9.04 | 12 | 62 | " " |
| 80. Smith..... | 24.9.04 | 22 | 29 | " " |
| 81. Rantiome..... | 24.9.04 | 14 | 24 | " " |
| 82. Kinsella..... | 25.9.04 | 16 | 30 | " " |
| 83. Anthony..... | 25.9.04 | 14 | 18 | " " |
| 84. Smith..... | 25.9.04 | 12 | 30 | " " |
| 85. Anthony..... | 26.9.04 | 16 | 19 | " " |
| 86. Smith..... | 26.9.04 | 16 | 31 | " " |

3. Examination of Sweat.

Critical perspirations, which are very characteristic of Mediterranean fever, have been examined at various periods of the disease, but the *M. melitensis* has not yet been isolated. The following examinations have been made:—

Experiment I.—On June 22, 1904, P . . . was noticed to be sweating profusely. The sweat was soaked up by means of sterile swabs which were then planted out in broth and rubbed over nutrose-agar plates

The tubes and plates were incubated at 37° C. On June 25, 1904, all the broth tubes showed a growth which was plated on nutrose-agar. The primary and secondary agar plates were carefully examined from time to time, but no signs of the *M. melitensis* could be discovered.

Experiment II.—At 8.30 P.M. on June 22, 1904, P . . . was again sweating profusely; swabs were treated as above, but the *M. melitensis* did not appear in the plates.

Experiment III.—At midnight on June 22, 1904, profuse sweats occurred in the same case, and the procedure detailed under Experiment I was followed. The *M. melitensis* was not isolated.

Experiment IV.—In the broth tubes, prepared as above, many contaminations were observed, which often rapidly overgrew the plates and so possibly prevented the *M. melitensis* from developing. In order to get rid of these extraneous organisms as far as possible the skin of P . . . was carefully washed with carbolic acid and ether, and a sterile pad covered by a sterile watch glass was bandaged on the right arm. On June 27, 1904, a critical sweat occurred, the pad was removed and planted out in broth; a growth occurred on June 29, 1904, which was found to consist of large Gram-staining cocci; no signs of the *M. melitensis* were discovered.

Experiment V.—On June 28, 1904, the procedure detailed under Experiment IV was followed in the case of H . . . large Gram-staining cocci again appeared.

Experiment VI.—On June 27, 1904, the same procedure was followed in the case of K . . . large and small Gram-staining cocci were isolated, but the *M. melitensis* did not appear.

Experiment VII.—On June 29, 1904, saturated pads obtained from P . . . were examined; the broth tubes remained absolutely sterile, although the incubation was continued for 10 days.

Experiment VIII.—On June 29, 1904, pads from Wildbore were planted out in broth. No growth resulted.

Experiment IX.—On June 29, 1904, pads from Wilson were planted out in broth. A growth occurred which, when plated, was found to give rise to large colonies, consisting of large cocci staining with Gram.

Experiment X.—On June 30, 1904, pads from Kelly were planted out in broth. No growth resulted.

It might be thought that the failure to obtain a growth recorded under Experiments VII, VIII, and X was possibly due to the presence in the swabs of carbolic acid, which, when transferred to the broth tubes, might inhibit the growth of the *M. melitensis*. In order to ascertain whether this was the case, sterile broth tubes, obtained in the manner detailed, were inoculated with *M. melitensis*. A typical growth resulted, showing that the failure to obtain a growth was not due to the presence of the disinfectant.

Experiment XI. Monkey No. 74.

To determine if the Injection of Sweat, from Malta Fever Patients, into a Monkey will give rise to the specific Fever.

The monkey arrived on August 29, 1904, and was taken at once to the roof of the Station Hospital, Valletta.

September 12, 1904. Skin scrapings were taken from the arms and axillæ of Private Lawrence, and ground up with normal salt solution. The resulting emulsion was injected subcutaneously into Monkey No. 74.

September 17, 1904. The blood was examined; the serum in a low dilution appeared to have a tendency to agglutinate the *M. melitensis*.

September 23, 1904. The blood was again examined, but the serum, diluted 1—10, did not show any signs of agglutinating the *M. melitensis*, even after waiting 1 hour.

September 25, 1904. Skin scrapings made into an emulsion with salt solution, were again injected.

September 27, 1904. Skin scrapings, treated as before, were injected.

September 28, 1904. The blood was examined, but the serum gave no reaction with the *M. melitensis*.

October 24, 1904. Staff-Surgeon Shaw continued the experiment up to this date. An agglutinative reaction was obtained with the serum, diluted 1—40, twenty-two days after the first injection.

The final result will be found in Dr. Shaw's experiments.

Experiment XII.

To determine if the Injection of Bacteria Free Sweat, derived from Malta Fever Patients, causes the Development of Agglutinins in the Blood of a Monkey.

Monkey No. 61A arrived in the laboratory on September 9, 1904. On September 15, 1904, and September 21, 1904, the serum was added in a low dilution to an emulsion of the *M. melitensis*; no trace of agglutination was observed.

September 22, 1904. Skin scrapings were taken from the arms and axillæ of Privates Kinsella and Silburn, who were suffering from Mediterranean Fever, and ground up with normal salt solution so as to form a fine emulsion. A sterile Berkefeld candle having been inserted into a sterile test-tube, the emulsion was filtered so as to remove all bacteria. The filtrate was then injected subcutaneously into Monkey No. 61A.

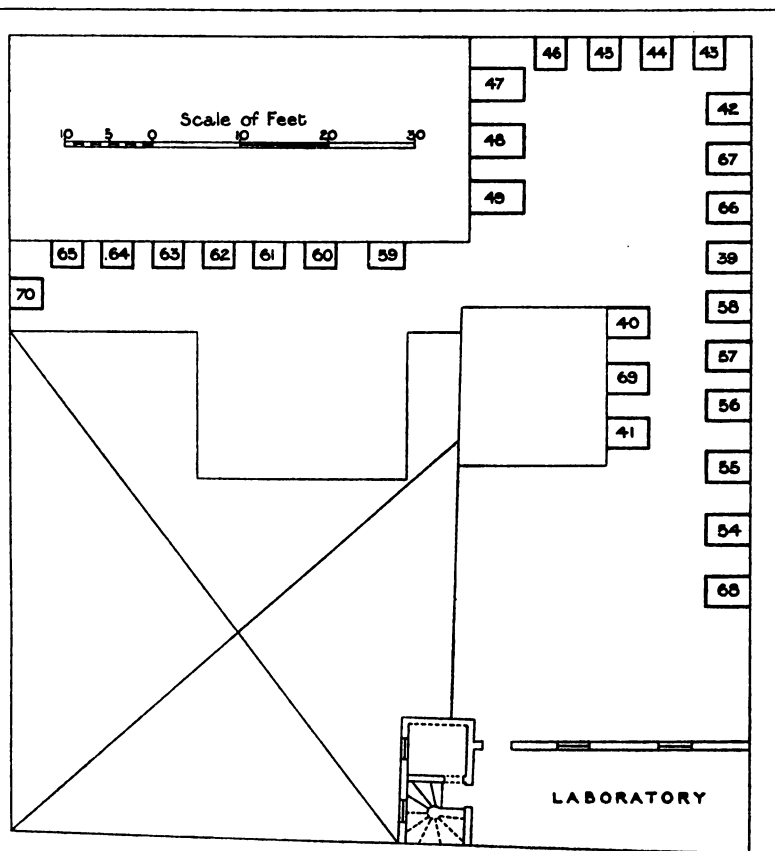
September 24, 1904. Sweat obtained from Privates Smith,

Silcocks, and Kinsella was similarly filtered, and the filtrate injected subcutaneously.

September 26, 1904. The blood was examined, and the serum found to have no action on the *M. melitensis*.

October 24, 1904. Dr. Shaw continued the experiment up to this date. The blood serum never caused the slightest agglutination of the *M. melitensis*.

Result.—The bacteria free filtrate obtained from the sweat of Malta fever patients does not appear to give rise to agglutinins in the blood of a monkey.



Plan of the Roof where Monkeys were kept, showing Position of the Animals which became naturally infected.

4. Examination of Expired Air of Malta Fever Patients.

In order to ascertain the presence of the *M. melitensis* in the expired air of Malta fever patients, a test-tube was fitted with an indiarubber

bung through which passed two glass tubes : one, attached to a mouth-piece, reached to the bottom of the test-tube and the other the exit tube, just passed through the bung. The test-tube was half-filled with nutrient broth and the whole apparatus then sterilised in the autoclave.

The patient under examination was directed to force expired air through the broth at frequent intervals throughout the day. The indiarubber bung, with glass tubes, was then removed, and the test-tube, being plugged with sterile cotton wool, was incubated at 37° C. After four days' incubation the broth was plated on nutrose-glucose-litmus-agar plates, and likely colonies fished and tested in the usual manner.

Case 1.—Private Markham breathed through one of these tubes on the 12.9.04; the tube was then incubated at 37° C. Four days later there was no sign of growth, but on the 19.9.04 a slight opalescence was noted. The broth was then plated on nutrose-glucose-litmus-agar. The plates were incubated for seven days, but no colonies of the *M. melitensis* appeared.

Case 2.—Private Lawrence breathed through a tube on the 12.9.04. On the 16.9.04 a marked growth appeared. A portion of the broth was plated as above, and the remainder of the growth injected into Monkey No. 73. After seven days' incubation no signs of *M. melitensis* could be discovered in the plates.

Case 3.—Private Markham again breathed through a tube on the 14.9.04. The tube was treated as before, and a slight growth was noticed on the 21.9.04. The growth was then plated, but no colonies of the *M. melitensis* appeared.

Case 4.—Private Lawrence breathed through a tube on the 14.9.04. On the 21.9.04 a slight growth appeared, which was then plated as before. No colonies of the *M. melitensis* were seen in the plates.

Case 5.—Private Kinsella breathed through a tube on the 17.9.04. On the 26.9.04 a slight growth appeared, but no colonies of *M. melitensis* were discovered in the plates made with the opalescent broth.

Case 6.—Private Silburn breathed through a tube on the 17.9.04. After twenty-four hours' incubation, the broth, being distinctly turbid, was plated in the usual manner, and incubation of the tube continued. Four days later a portion of the growth in the test-tube was plated out and the remainder of the growth injected into Monkey No. 73. No signs of the *M. melitensis* were discovered in the plates after prolonged incubation at 37° C.

Case 7.—Private Kinsella again breathed through a tube on the 20.9.04. No growth appeared in the broth, though incubation was continued for fourteen days.

Case 8.—Private Silburn breathed through a tube on the 20.9.04. A marked growth, having a putrefactive odour, appeared on the 24.9.04. This was then plated out as usual, but no colonies of the *M. melitensis* were discovered.

Case 9.—Private Silburn again breathed through a tube on the 23.9.04. The growth which appeared after incubation was treated in the usual manner, but no colonies of *M. melitensis* were isolated.

Case 10.—Private Tripp breathed through a tube on the 23.9.04. The tube was plated as before, but the *M. melitensis* was not isolated.

Case 11.—Private Anthony breathed through a tube on the 23.9.04. After the usual incubation the resulting growth was plated out, but with a negative result.

Case 12.—Private Rivers breathed through a tube on the 23.9.04. After the usual treatment, the *M. melitensis* was not isolated.

Monkey No. 73.

This monkey was reserved for the injection of broth infected by the expired air of Malta fever patients.

The monkey arrived at the laboratory on the 8.9.04. On the 15.9.04 a portion of its blood was removed and the serum, in a low dilution, added to an emulsion of the *M. melitensis*. No traces of agglutination were observed. On the 16.9.04 10 c.c. of broth infected by the breath of Private Lawrence were injected subcutaneously. On the 21.9.04 10 c.c. of broth infected by the breath of Private Silburn were injected. The action of the blood serum on the *M. melitensis* was also tested on this day, but no signs of agglutination were observed. On the 28.9.04 the blood serum was again examined, but no reaction with the *M. melitensis* was observed, though the dilution of the serum was only 1—10.

5. *Examination of Sea-water in the Grand Harbour, Malta.*

Having in view the result obtained when studying the viability of the *M. melitensis* in sea-water, and the fact that sea-water is extensively used for washing the decks of the battleships stationed in the Grand Harbour, it appeared desirable to ascertain whether the *M. melitensis* could be discovered in sea-water taken from this locality.

Studies of sea-water, when unsterilised and grossly infected with the *M. melitensis*, soon showed that the specific microbe could not be isolated, by ordinary bacteriological methods, a few days after the infection, owing to the saprophytic organisms overgrowing the colonies of the *M. melitensis*. Accordingly, it was decided to filter the sea-water through a sterile Berkefeld candle, and after washing the deposit with tap-water, to suspend it in 10 c.c. of tap-water, and inject the whole subcutaneously into a monkey.

On the 9.9.04 600 c.c. of sea-water, taken from the Grand Harbour opposite Fort St. Angelo, were pumped through a Berkefeld candle, and the deposit, having been well washed, was diffused in 10 c.c. of tap-water and injected subcutaneously into Monkey No. 71.

On the 10.9.04, the deposit from 600 c.c. of sea-water, taken from the same place, was injected.

On the 13.9.04, the deposit from 600 c.c. of sea-water, taken as before, was injected.

On the 15.9.04 the same procedure was followed.

On the 17.9.04 the same procedure was followed.

On the 18.9.04 the serum of Monkey No. 71 was added to an emulsion of the *M. melitensis*. No traces of agglutination were observed.

On the 19.9.04 600 c.c. of sea-water, taken off Fort St. Angelo, were again filtered, washed, and injected.

On the 21.9.04 the same procedure was followed.

On the 23.9.04 the same procedure was followed.

On the 25.9.04 1800 c.c. of sea-water were treated as before and the deposit injected. The serum of the monkey was added to an emulsion of *M. melitensis*, but no reaction was obtained.

On the 27.9.04 1800 c.c. of sea-water were filtered, and the washed deposit injected.

On the 29.9.04 600 c.c. of sea-water were treated as before. There is a small abscess at the site of the inoculation of the 27th.

Dr. Shaw continued this experiment up to October 22; the monkey received the bacteria contained in 30 litres of sea-water, but the blood serum never caused the slightest agglutination of the *M. melitensis*.

Result.—The *M. melitensis* could not be detected in the sea-water of the Grand Harbour.

EXPERIMENTS ON THE MODE OF CONVEYANCE OF THE *MICROCOCCLUS MELITENSIS* TO HEALTHY ANIMALS.

By Major W. H. HORROCKS, R.A.M.C., Member Mediterranean
Fever Commission.

(Received September 17, 1904.)

Experiment I.—Monkey No. 41.

*To Determine if the Inhalation of Dust, Infected with M. melitensis, will
give Rise to Mediterranean Fever in Healthy Monkeys.*

July 10, 1904. Monkey placed in cage and infected dust blown round him. Dust in bottle A used for this experiment, infected July 2, 1904.

July 11, 1904. Monkey kept in the cage and dust again blown round him. It was noticed, however, that owing to the moisture condensed on the walls, the dust soon settled, and it was impossible to keep it passing backwards and forwards through the cage. After an hour's interval, the cage was opened and the monkey allowed to come out into the room. Cage was then disinfected and dried.

July 12, 1904. Same procedure as July 10, 1904.

| | | |
|---------|-----------------|-----------------------------------|
| " 13, " | " | " |
| " 14, " | " | " |
| " 15, " | " | " |
| " 16, " | " | " |
| " 18, " | " | " |
| " 19, " | " | " |
| " 20, " | " | " |
| " 21, " | " | " |
| " 22, " | Tested blood. | No reaction. |
| " 23, " | Placed in cage; | dust blown as before. |
| " 25, " | Placed in cage. | The dust (bottle A) all expended. |

Planted out one loop in broth to try and determine presence of *M. melitensis*. July 26, 1904, growth planted on agar; no signs of *M. melitensis*.

July 25, 1904. Prepared more dust to-day ; dust (Petri dish half full) sterilised, and then inoculated with four agar slopes, third generation from spleen of man, dried over sulphuric acid *in vacuo*.

July 29, 1904. Monkey placed in cage and dust blown as before ; dust dried over sulphuric acid employed.

July 31, 1904. Monkey placed in cage and dust blown as before ; dust dried over sulphuric acid employed.

Note.—The dust appears to fall very rapidly ; only seen on the nostrils. Mouth, as a rule, kept tight shut.

August 1, 1904. The same procedure as on July 29, 1904.

“ 2, “ “ “

August 3, 1904. The same procedure as on July 29, 1904. Planted out soil in broth to see if *M. melitensis* still present ; growth August 6, 1904, planted on agar. *M. melitensis* recovered.

August 4, 1904. The same procedure as on July 29, 1904 ; dust all expended.

August 5, 1904. Fresh dust prepared. Four tubes, second generation, spleen of Howe, incubated 3 days at 37° C., dried 24 hours over sulphuric acid and CaCl_2 *in vacuo*. Dust blown in cage. Dust planted out in broth on August 4, 1904, to ascertain presence of *M. melitensis*. August 8, 1904, planted on glucose agar ; no *M. melitensis*, isolated ; broth probably contaminated. This batch of broth found to be contaminated with *B. mesentericus*.

August 6, 1904. Dust blown in cage as on August 5, 1904. Dust planted out in broth August 9, 1904. Growth planted on agar August 10, 1904 ; broth contaminated, cause probably as on August 4, 1904.

August 8, 1904. Dust blown as before.

August 9, 1904. Planted out dust in broth (proved by incubation). On August 13, 1904, growth planted on glucose-litmus-agar, *M. melitensis* present.

August 10, 1904. Dust blown as before.

August 16, 1904. Examined blood ; serum gave no reaction with *M. melitensis* in a dilution of 1 in 10.

August 26, 1904. Examined blood ; serum reacted at once with *M. melitensis* in a dilution of 1 in 20 ; no reaction 1 in 50.

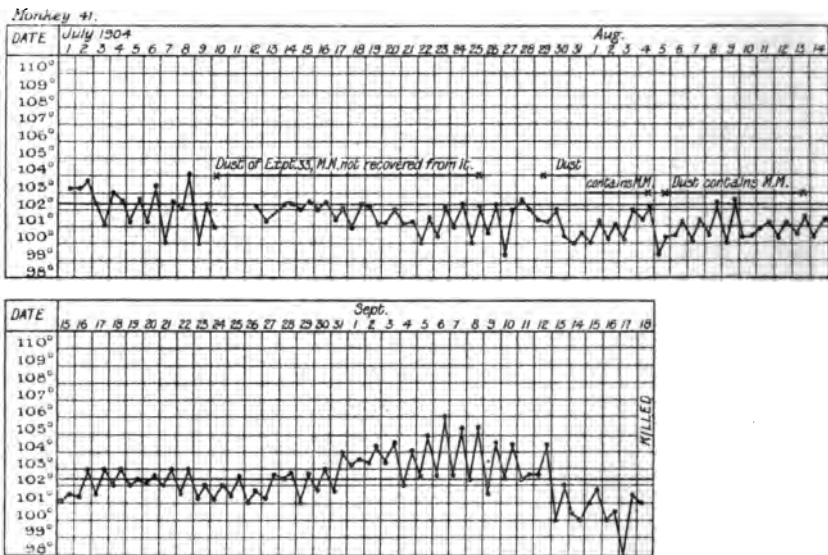
September 6, 1904. Examined blood ; serum reacted at once, visible to naked eye, dilution 1—100 ; no reaction 1—500.

September 15, 1904. Examined blood ; serum reacted at once, visible to naked eye, dilution 1—50 ; reaction incomplete in a dilution of 1—100.

September 19, 1904. Killed the monkey with chloroform. *Post-mortem* examination : Spleen enlarged, soft, and friable. Liver and kidneys congested. Made cultures from spleen, liver, and kidneys, urine, and heart's blood.

September 23, 1904. *M. melitensis* isolated from spleen of this monkey. Cultures made from liver, kidneys, and heart's blood are sterile.

The following chart represents the course of the rectal temperature :



Monkey No. 41.

Note.—The wave of fever did not commence until August 31, though a slight serum reaction was obtained on August 26. The first date on which the dust was known to contain the *M. melitensis* was July 29, consequently the incubation period might have varied from 17 to about 30 days.

Result.—This experiment seems to show that the inhalation or ingestion of infected dust will give rise to the disease.

Experiment II.—Monkey No. 47.

To determine if the Injection of Dust, infected with M. melitensis, into the Nostrils and Throat will give rise to Mediterranean Fever in Healthy Monkeys.

July 9, 1904. Injected dry dust containing *Micrococcus melitensis*, 7 days old, into both nostrils of above monkey. (Bottle A of July 2, 1904, used—Experiment 33.)

July 10, 1904. Injection repeated.

„ 11, „ „
„ 12, „ „

July 13, 1904. Injection repeated.

" 14, " "

" 21, " Examined blood; no reaction with *M. melitensis*.

" 28, " "

" 29, " Injected infected dust, dried 2 days over sulphuric acid *in vacuo*, into back of throat; lips covered with a cloth, and tube passed through a wooden gag.

July 30, 1904. Injection repeated as on July 29, 1904.

August 1, " "

" 2, " "

" 3, " "

" 4, " "

" 5, " Injection repeated, fresh dust prepared from four agar slopes, spleen Howe, second generation, incubated 3 days at 37° C., then dried for 24 hours over sulphuric acid and calcium chloride *in vacuo*.

August 6, 1904. Injection repeated as on August 5, 1904.

" 8, " Injection repeated. The greatest care is being taken to prevent abrasions of the mucous membrane; a wooden gag is inserted between the teeth as before.

August 9, 1904. Examined blood; serum reacts completely to naked eye, dilution 1—40; slight reaction 1—80. No abrasions to be seen in the mouth; on the skin of the lower lip there is a very small abrasion, caused by the gag, but it is unlikely that this was the source of infection, as the lips have been covered as much as possible when the dust was blown.

August 16, 1904. Complete reaction at once 1—100, naked eye; slight reaction 1—300.

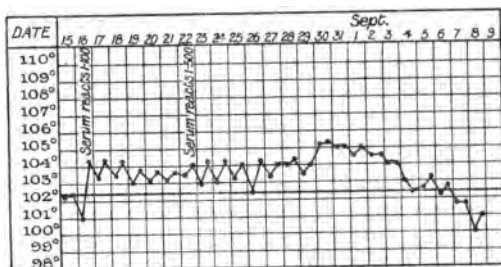
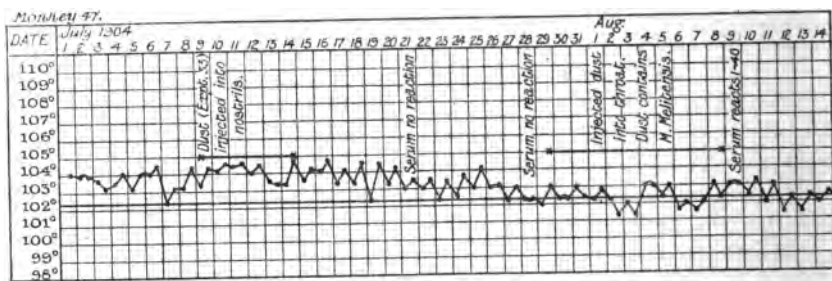
August 22, 1904. Examined blood, complete reaction at once 1—200; complete reaction, visible to naked eye in 10 minutes, dilution 1—500; 1—1000 dilution, *nil*.

September 9, 1904. This monkey has been very ill for some days, and has lost flesh rapidly. Being obviously in a dying state, he was killed with chloroform this morning. *Post-mortem* examination: Spleen enlarged, soft and friable. Kidneys markedly congested. Liver congested. Pericardium contained some fluid. Other viscera healthy.

Made cultures from the spleen, kidneys and liver.

The *M. melitensis* was not recovered, as all the cultures proved to be contaminated. The monkey was dying, and a batch of broth, which had not been tested by incubation, had to be used; unfortunately, all the broth tubes were found, on incubation, to be contaminated by *B. mesentericus*.

The following chart represents the course of the temperature:—



Monkey killed
September 9.

Monkey No. 47.

Result.—From this and the last experiment it is evident that the inhalation or swallowing of infected dust will give rise to Mediterranean Fever in monkeys.

Experiment III.—Monkey No. 39.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

This monkey was kept under observation from July 1—10, 1904. It appeared perfectly healthy, and no cuts or abrasions were visible either on the body or in the mouth.

July 10, 1904. The growth from one agar slope, second generation, from spleen of man, and grown for 7 days at 37° C., was mixed with boiled potato, and eaten by the monkey.

July 11, 1904. The growth from one agar slope, as above, but grown for 8 days at 37° C., was mixed with boiled potato and two plums, and eaten by the monkey.

July 12, 1904. The same procedure followed, but the agar slope was 9 days old.

July 13, 1904. As on the 12th; growth 10 days old.

July 14, 1904. The same procedure followed, but a 9 days' old culture from heart's blood of a rabbit was employed.

July 15, 1904. Ten days' old culture, third generation, spleen of man used.

July 16, 1904. The same as on the 15th.

„ 18, „ „ „

„ 19, „ Feeding continued as on July 15, 1904.

„ 20, „ Feeding continued as on July 15, 1904. Examined blood, serum diluted 1—10, gave no reaction with the laboratory strain of *M. melitensis*.

July 21, 1904. Feeding continued. One agar slope, first generation, spleen H—, incubated for 7 days at 37° C., used.

July 22, 1904. Feeding continued. One agar slope, first generation, kidney H—, incubated for 8 days at 37° C., used.

July 23, 1904. The feeding was continued, but I omitted the plums from the mixture, as I found they gave rise to a strongly acid reaction which might inhibit or destroy the *M. melitensis*. One agar slope, first generation, kidney of H—, incubated for 9 days at 37° C., was employed.

July 25, 1904. Half an agar tube of third generation, spleen of H—, was given. The blood was examined for agglutination, but the serum, diluted 1—10, gave no reaction with the *M. melitensis*.

July 26, 1904. Half an agar tube of third generation, spleen of H—, incubated for 4 days at 37° C., was employed.

July 27, 1904. One agar slope, third generation, spleen of H—, incubated for 14 days at 37° C., mixed with potato.

July 28, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

July 29, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

July 30, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 1, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 2, 1904. One agar slope, fifth generation, spleen of H—, incubated for 5 days at 37° C., mixed with potato.

August 3, 1904. One agar slope, second generation, spleen of H—, incubated for 3 days at 37° C., mixed with potato. Only a small portion was consumed.

August 4, 1904. One agar slope, fourth generation, spleen of H—, incubated for 5 days at 37° C. Only a small portion was eaten.

August 5, 1904. One agar slope, second generation, from urine of Sergeant P—, and incubated 10 days at 37° C., mixed with potato.

August 6, 1904. One agar slope, third generation, spleen of H—, incubated for 72 hours at 37° C., mixed with potato.

August 8, 1904. One agar slope, third generation, spleen of H—, incubated for 6 days at 37° C., mixed with potato.

August 9, 1904. One agar slope, third generation, spleen of H—, incubated for 6 days at 37° C., mixed with potato.

August 10, 1904. One agar slope, third generation, spleen of H—, incubated for 15 days at 37° C., mixed with potato. Examined blood; serum reacts at once, visible to the naked eye, in a dilution of 1—80; under the microscope reaction is seen at once with a dilution of 1—160.

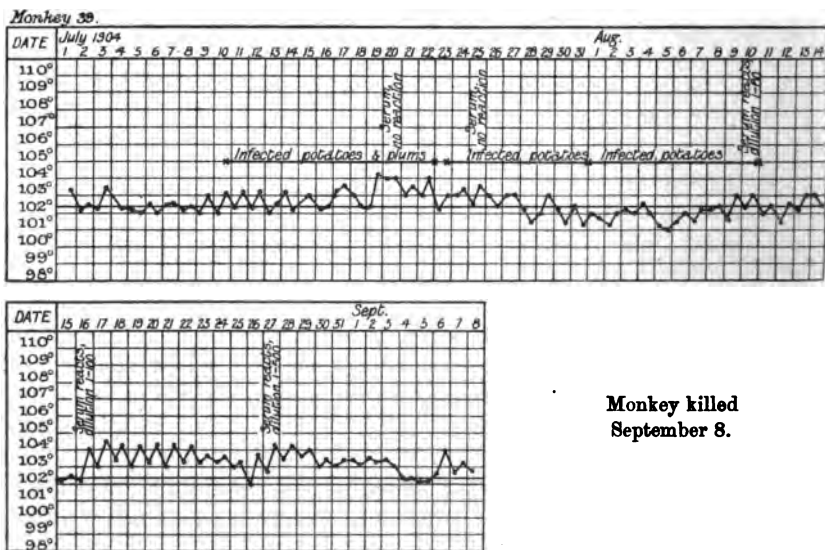
August 16, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. Dilution 1—300 shows a reaction under $\frac{1}{12}$ th.

August 27, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. After 5 minutes, dilution 1—500, is visible to the naked eye.

September 8, 1904. Killed the monkey with chloroform. Body well nourished. *Post-mortem*. Spleen enlarged, soft and friable. Kidneys congested. Liver congested. Other viscera normal.

September 14, 1904. Recovered *M. melitensis* from the spleen.

The following chart represents the temperature curve:—



Monkey No. 39.

Result.—The absorption of the *M. melitensis* was extremely slow, but the monkey eventually suffered from an acute infection.

Experiment IV.—Monkey No. 40.

To Determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

July 10, 1904. Half of the potato prepared for Monkey No. 39 was given to this monkey. The dose of *M. melitensis* corresponded to one agar slope, as in the case of Monkey No. 39.

July 11, 1904. The same procedure was followed as in Experiment III, Monkey No. 39.

| | | | | | |
|---|-----|---|---|---|---|
| " | 12, | " | " | " | " |
| " | 13, | " | " | " | " |
| " | 14, | " | " | " | " |
| " | 15, | " | " | " | " |
| " | 16, | " | " | " | " |
| " | 18, | " | " | " | " |
| " | 19, | " | " | " | " |
| " | 20, | " | " | " | " |
| " | 21, | " | " | " | " |
| " | 22, | " | " | " | " |
| " | 23, | " | " | " | " |
| " | 25, | " | " | " | " |

The same procedure was followed as in Experiment III, Monkey No. 39. Examined blood ; serum gave no reaction with *M. melitensis*.

" 26, " The same procedure was followed as in Experiment III, Monkey No. 39.

| | | | | | |
|---|-----|---|---|---|---|
| " | 27, | " | " | " | " |
| " | 28, | " | " | " | " |
| " | 29, | " | " | " | " |
| " | 30, | " | " | " | " |

August 1, " " " " "

" 2, " " " " "

" 3, " " " " "

" 4, " " " " "

" 5, " " " " "

" 6, " " " " "

" 8, " " " " "

" 9, " " " " "

" 10, " " " " "

" 11, " " " " "

Examined blood. Complete instantaneous agglutination, visible to the naked eye, dilution 1—30. After standing 5 minutes, dilution 1—100 ; was also visible to the naked eye.

August 20, 1904. Examined blood. Serum gave a reaction with *M. melitensis* when diluted 1—10, but no result was obtained with higher dilutions.

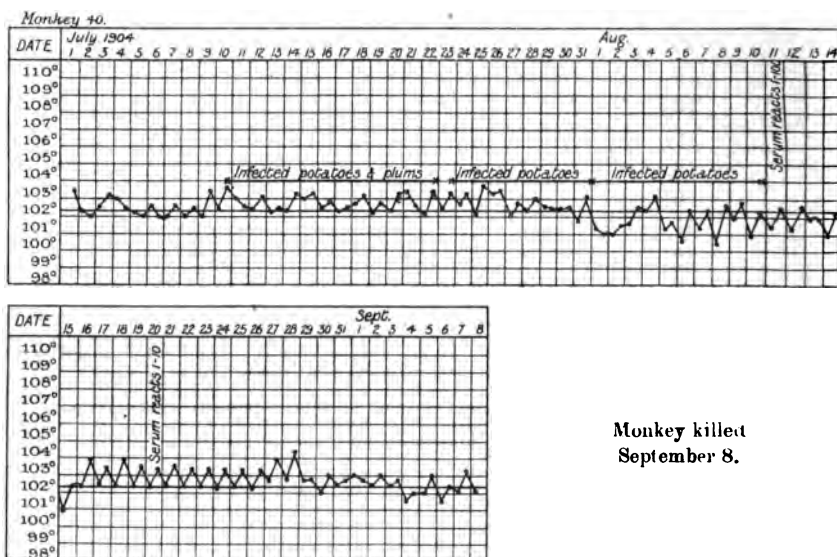
September 8, 1904. Monkey killed by chloroform. *Post-mortem*

Spleen enlarged, but not so markedly as No. 39; kidneys congested; other viscera apparently healthy. Made cultures from the spleen, liver, and kidneys.

September 16, 1904. *M. melitensis* not recovered from the cultures made at the *post-mortem* examination. All the cultures proved to be sterile.

Note.—It seems probable that, in the case of this monkey, the bacterial infection was never marked, and that the few micrococci absorbed might easily have been destroyed.

The following chart represents the temperature curve:—



Monkey killed
September 8.

Monkey No. 40.

Experiment V.—Monkey No. 66.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

August 13, 1904. This monkey is in a box next to Monkey No. 39, and I noticed about a week ago that he ate some of the infected potato provided for No. 39. Examined blood, serum reacts instantaneously, visible to naked eye, dilution 1—100. Visible under $\frac{1}{2}$ th after 10 minutes in a dilution of 1—500.

August 18, 1904. Believing this monkey to be healthy, Dr. Zammit, at 6.30 P.M. last evening, injected a small quantity of blood from a Mediterranean Fever patient. In order not to vitiate both experiments the monkey was killed at 11 this morning.

Post-mortem examination:—

Abdomen: Spleen enlarged and congested. Kidney enlarged and congested. Liver congested. Intestines appeared normal.

Thorax: Lungs healthy. Heart appeared dilated.

Cultures made as follows:—

- Spleen: (a) Planted out in broth and (aa) rubbed over an agar slope.
 (b) Kidney planted out in broth and (bb) rubbed over an agar slope.
 (c) Liver planted out in broth.
 (d) Heart's blood, planted out in two broth tubes.
 (e) Urine, planted out in broth.

August 21, 1904. Typical colonies have appeared on the agar slope, made from the spleen; fished one—it agglutinated at once with serum from Monkey No. 45.

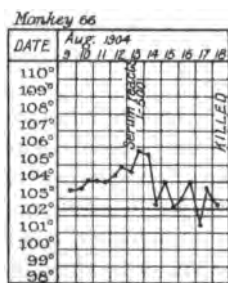
- August 22, 1904. Planted out colony from spleen on an agar slope.
 „ growth in broth, from heart's blood (two tubes), on an agar slope.
 „ growth in broth, from kidney, on an agar slope.
 „ growth in broth, from liver, on an agar slope.

August 24, 1904. Typical growth obtained from colony of spleen, planted out in litmus milk and glucose. Litmus milk rendered alkaline, glucose not fermented.

August 26, 1904. Typical growth, agglutinating at once with dilute serum, obtained from heart's blood.

August 28, 1904. Typical growth, agglutinating at once with monkey serum, obtained from kidney.

The following chart represents the temperature curve:—



Monkey No. 66.

Note.—This experiment is probably an instance of direct absorption of *M. melitensis* through a crack or abrasion of the mucous membrane of the mouth. The period of incubation and the wave of fever correspond exactly with those of Monkey No. 72, which was infected with

M. melitensis through a crack in the mucous membrane over the incisor teeth.

Experiment VI.—Monkey No. 72.

To Differentiate between Absorption from the Mouth and Throat and Absorption from the Stomach and Intestines.

Monkey No. 72 arrived in the laboratory on September 10, 1904. The blood was tested and gave no reaction with the *M. melitensis*. Feeding was then commenced, infected milk being passed directly into the stomach by means of an indiarubber tube. The growth on one agar slope, second generation, spleen of H—, incubated for 6 days at 37° C., was employed.

September 13, 1904. The feeding was continued as before, the growth on one agar slope, incubated for 7 days, being given. A small quantity of the milk regurgitated into the mouth, but no abrasion could be seen on the mucous membrane.

September 14, 1904. The growth from one agar slope, incubated for 8 days, was given.

September 15, 1904. The feeding was continued as before.

September 16, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given. A little milk again regurgitated into the mouth, and, on examination, a small crack was found in the mucous membrane opposite the upper incisor teeth. The mouth was at once washed out with lysol. The blood was examined but no reaction with the *M. melitensis* was obtained.

September 17, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given.

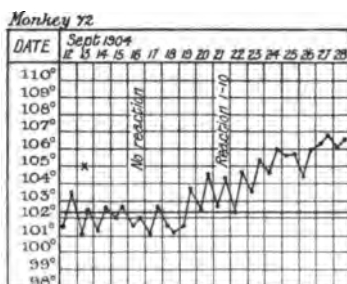
September 18, 1904. The growth from one agar slope, incubated for 6 days, was given.

September 21, 1904. The blood was examined and the serum in a dilution of 1—10, caused instantaneous clumping of the *M. melitensis*.

September 26, 1904. The serum, diluted 1—100, was found to agglutinate the *M. melitensis* instantaneously, the reaction being visible with the naked eye.

Note.—This monkey was directly infected either on September 13 or 16, the short incubation and sharp rise of temperature correspond to what is seen when the *M. melitensis* is directly absorbed into the peripheral circulation. Owing to the regurgitation of the infected milk into the mouth the experiment failed to differentiate between absorption from the mouth and from the alimentary canal; it, however, explains what probably occurred in the case of Monkey No. 66.

The prolonged incubation or rather slow absorption observed in the case of Monkeys Nos. 39, 40, and 41 forms a marked contrast to the rapid infection noticed in Monkeys Nos. 66 and 72, and approximates very closely to the results obtained when human beings are infected under natural conditions.



Monkey No. 72.

Experiment VII.—Monkey No. 45.

To note the Effect of the Subcutaneous Inoculation of *M. melitensis* in Healthy Monkeys, and to Obtain a Specific Serum.

July 9, 1904. Injected $\frac{1}{2}$ c.c. of emulsion from an agar tube, second generation, from spleen of man. The agar tube was incubated for 6 days at 37° C., and the whole of the growth was used for the emulsion.

July 15, 1904. Complete agglutination with *M. melitensis* serum, diluted 1—10, and up to 1—160. No reaction with a dilution of 1—300.

July 21, 1904. Monkey looks ill. Tested serum—complete reaction, naked eye at once, dilution 1—1000. Shaved hair on back, and Zammit applied two female *Stegomyia*, which fed voraciously.

July 22, 1904. Zammit's feeding experiments with mosquitoes continued.

July 23, 1904. Zammit's feeding experiments with mosquitoes continued.

July 26, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

August 1, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

August 3, 1904. Monkey suffering from rheumatism (?); right arm and right wrist joint painful.

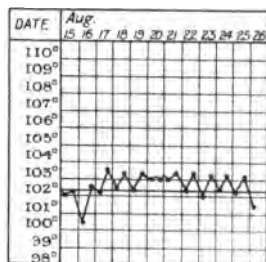
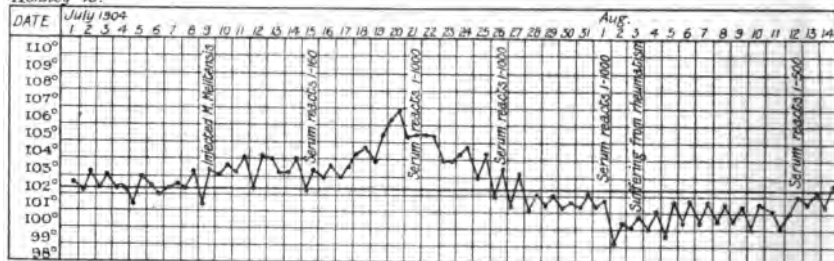
August 12, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—100; reaction after 5 minutes, dilution 1—500; dilution 1—1000, no reaction 5 minutes; feeble reaction, under microscope, after $\frac{1}{2}$ hour.

September 9, 1904. Killed the monkey with chloroform. *Post-mortem*: Spleen much enlarged. Liver and kidneys congested. Other viscera healthy. Made cultures from the spleen, kidney and liver.

September 13, 1904. Recovered *M. melitensis* from spleen.

The following chart represents the temperate curve:—

Monkey 45.



Monkey No. 45.

Result.—The monkey suffered from a typical attack of Mediterranean fever.

Experiment VIII.—Monkey No. 48.

To Note the Effect of the Injection of Washings of Dust derived from Sergeants' Mess, Melleha Camp.

July 16, 1904. Dried soil (dust) from ventilation aperture, between w.c. and dining room of sergeants' mess at Melleha, received from Dr. Johnstone.

Soil macerated in sterile water, filtered, soil remaining washed, filtrate treated as follows:—10 c.c. injected into Monkey No. 48, subcutaneously between shoulders.

July 18, 1904. Ten cubic centimetres of further washings injected.

July 23, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 11, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 26, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

This experiment was performed at the request of Dr. Johnstone. The sergeants' mess at Melleha appeared to be the probable centre of infection of the sergeants of the Essex regiment. A disused w.c. was

found communicating by a ventilating aperture with the mess room. The dust was derived from this ventilating aperture.

The following chart represents the temperature curve :—



Monkey No. 48.

Result.—The *M. melitensis* was not present in the dust removed from the ventilating aperture.

Experiment IX.—Monkey No. 43.

To Note the Effect of the Injection of Washings of Supposed Infected Soil into a Healthy Monkey.

July 16, 1904. Dr. Johnstone forwarded 0·14 gramme of soil, obtained from the pan of the disused w.c. in the sergeants' mess, Melleha Camp. The soil was macerated in sterile water, filtered through paper, and the deposit again thoroughly washed. The total filtrate obtained was 20 c.c. Of this 10 c.c. was injected subcutaneously into a monkey.

July 18, 1904. The remainder of the washings injected.

July 23, 1904. Examined blood; serum, diluted 1—10, gave no reaction with *M. melitensis*.

| | | | | | |
|-----------|-----|---|---|---|---|
| August | 1, | " | " | " | " |
| " | 10, | " | " | " | " |
| " | 17, | " | " | " | " |
| " | 26, | " | " | " | " |
| September | 6, | " | " | " | " |

The following chart represents the temperature curve:—



Monkey No. 43.

Result.—The *M. melitensis* was not present in the soil removed from the w.c. in the sergeants' mess.

Experiment X. Monkey No. 46.

Injection of Washings from Wall of an Infected House.

July 7, 1904. The walls of the w.c., No. 26 Strada Nuova, Sliema, where two cases of Mediterranean Fever occurred, were rubbed with cotton wool moistened with saline solution; the water was expressed and filtered through paper. Filtrate, collected in a sterile tube, was treated as follows:—

Injected 10 c.c. of filtrate.

July 8, 1904. Injected 10 c.c. of filtrate.

July 9, 1904. " "

July 10, 1904. Injected the remaining portion (8 c.c.) of filtrate.

July 16, 1904. Examined blood, no reaction with *M. melitensis*, dilution 1—10.

July 18, 1904. Washings from kitchen, grown in broth for 11 days, injected to-day.

July 26, 1904. Tested serum, no reaction with *M. melitensis*, dilution 1—10.

August 10, 1904. Examined serum, no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood; serum reacts at once with *M. melitensis*, dilution 1—10; reaction 1—500, after waiting 15 minutes.

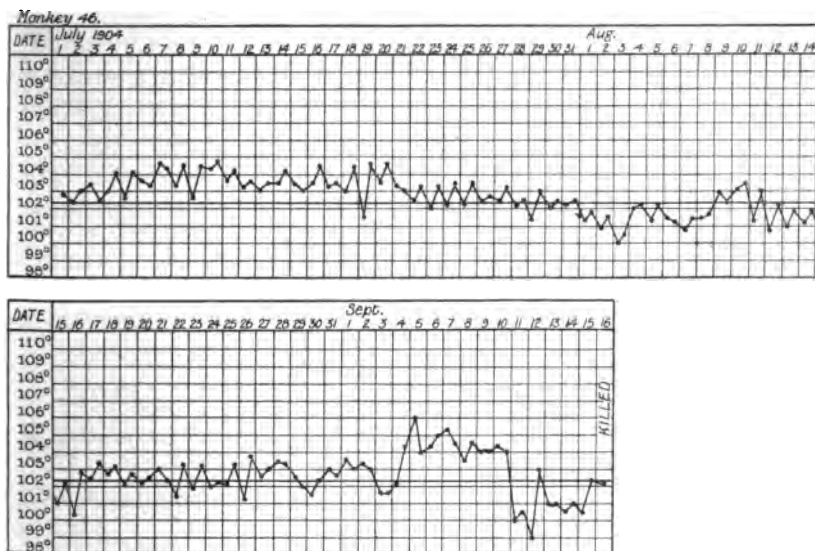
September 15, 1904. Examined blood; serum reacts at once in a dilution of 1—500; in a dilution of 1—1000 a reaction, visible to the naked eye, is seen in 5 minutes.

September 16, 1904. Killed the monkey with chloroform.

Post-mortem.—Spleen enlarged, but firm in consistence; kidneys and liver congested; pericardium contained a little fluid; other viscera healthy. Cultures made from spleen, liver, kidney, heart's blood, and urine.

September 23, 1904. *M. melitensis* isolated from spleen, kidney, and urine.

The following chart shows the temperature curve:—



Monkey No. 46.

Remarks.—This result is probably due to infection conveyed from neighbouring monkeys. Even if the *M. melitensis* had been present in the growth injected on July 18, it is highly improbable that the specific microbe when injected subcutaneously would have remained latent for a period of 50 days. Monkey No. 69 has also become infected since its arrival, without receiving the specific microbe either by the mouth or subcutaneously.

Monkey No. 46 on one side is next to Monkey No. 45, which received *M. melitensis* subcutaneously and developed a typical attack of fever.

On the other side of Monkey No. 46 is Monkey No. 47, infected by dust blown into the throat. Evidently this monkey has become infected, either by personal contact, by urine, or by means of *Stegomyia*.

Experiment XI.—Monkey No. 42.

To Determine if the Subcutaneous Injection of Infected Urine from a Case of Mediterranean Fever will give rise to the Disease in a Monkey.

July 13, 1904. Injected 10 c.c. of Howe's urine, enriched with broth, and incubated for 14 days at 37° C. (3 c.c. urine).

July 14, 1904. Injected 10 c.c. of Howe's urine (3 c.c. urine) treated as above, but incubated 15 days.

July 15, 1904. Injected 10 c.c. of mixed urine and broth (3 c.c. of urine), incubated 14 days.

July 18, 1904. Examined blood. Feeble reaction with one culture, blood diluted 1—10; tested with another culture, no reaction was obtained.

July 19, 1904. Injected 5 c.c. of broth culture, made at *post-mortem* of Howe by adding 1 c.c. of urine from bladder to broth, and then incubating at 37° C. for 12 days. Examined by hanging drop; fine cocci and chains, corresponding in morphology to *M. melitensis*, observed, the cocci decolorised by Gram.

July 20, 1904. Injected 10 c.c. of broth culture, made at *post-mortem* by adding contents of right ureter to a broth tube.

July 25, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 2, „ „ „ „

„ 11, „ „ „ „

„ 26, „ Examined blood; reacts 1—10 at once.

September 7, 1904. Examined blood; serum reacts in dilution of 1—20 at once: dilution 1—100, no reaction.

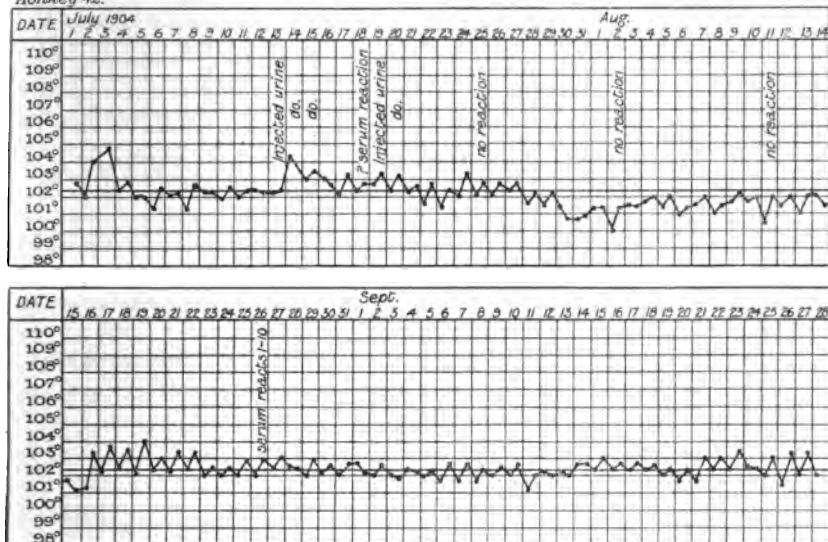
September 27, 1904. Killed monkey; made cultures from spleen, liver, kidney, heart's blood, and urine.

October 10, 1904. All the cultures have remained sterile.

Note.—The *M. melitensis* was recovered by plating another sample of the urine, removed from the bladder at the *post-mortem*.

The following chart shows the temperature curve; it will be noticed that there has never been a wave of fever, the slight serum reaction was probably caused by toxins contained in the urine:—

Monkey 42.



Monkey No. 42.

Remarks.—The *M. melitensis* was probably not present in the specimens of urine injected into this monkey. The slight blood reaction obtained might be caused by toxins in the urine.

Experiment XII.—Monkey No. 55.

To Determine whether Cultures of M. melitensis, Derived from Infected Urine, will give Rise to the Disease in a Monkey.

July 29, 1904. Growth from Pudney's urine, third generation, grown for 3 days on agar slope (glucose-litmus-nutrose-agar). The whole of the growth diffused in 2 c.c. of broth, and injected into this monkey.

August 4, 1904. Examined blood ; complete instantaneous reaction, visible to naked eye, blood dilution 1—10 ; no reaction 1—50.

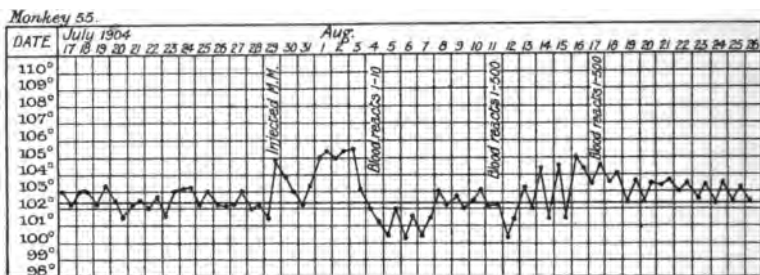
August 11, 1904. Examined blood ; complete instantaneous reaction visible to naked eye, dilution 1—100 ; after 5 minutes, reaction visible in dilution 1—500.

August 17, 1904. Examined blood ; reaction as on August 11, 1904.

September 8, 1904. Killed the monkey to-day. *Post-mortem* : Spleen enlarged and friable. Kidneys congested. Other viscera apparently healthy. Made cultures from the spleen, liver and kidneys.

September 12, 1904. *M. melitensis* recovered from the spleen.

The following chart shows the temperature curve ;—



Monkey No. 55.

Result.—This experiment shows that the *M. melitensis* recovered from the urine of Mediterranean Fever patients is capable of giving rise to the disease in healthy monkeys.

Experiment XIII.—Monkey No. 69.

Is Mediterranean Fever Conveyed from Diseased to Healthy Monkeys by Contact?

This monkey arrived in the laboratory on August 7, 1904. It was placed in a cage, between Monkey No. 41, infected by dust, and Monkey No. 40, infected by feeding. Monkey No. 69 appeared perfectly healthy on arrival, and ate well; its temperature was taken from August 9, and after August 16 displayed an erratic course, probably accounted for by the intense heat of the terrace from early morning until evening.

On August 26, 1904, the blood was examined, but the serum, diluted 1—10, gave no signs of reaction with *M. melitensis*.

On September 7, 1904, the blood was again examined, and the serum, diluted 1—10, caused immediate clumping of the *M. melitensis*, visible to the naked eye.

Since September 9, 1904, the monkey has been obviously ill, losing flesh and sitting "moping" in his box all day.

On September 11, 1904, the serum, diluted 1—20, caused instantaneous clumping of the *M. melitensis*.

On September 13, 1904, the monkey died, much emaciated.

Post-mortem examination: All the viscera appeared healthy; cultures were made from the spleen, liver, kidneys, heart's blood and urine. *M. melitensis* isolated from the spleen and liver.

Remarks as to the Mode of Infection of this Monkey.—It seems possible that it might have occurred in three ways, *i.e.* (a) by direct personal contact; (b) by direct infection from walking in the infected urine of his neighbours; (c) by means of *Stegomyia*. When at full

length of his chain, Monkey No. 69 could touch either of his neighbours and walk on the ground infected by them.

If personal contact alone had been the cause of infection, Monkey No. 48 ought to have been infected by Monkey No. 47. Also the *M. melitensis* has not yet been isolated from the sweat or skin scrapings of patients suffering from Mediterranean fever.

If the infection had been carried by *Stegomyia*, there should have been a general infection amongst the monkeys on the terrace. There appears no reason why mosquitoes should have picked out Monkey No. 69 and Monkey No. 46, which also appears to have been infected by its neighbours. At this time there were six other healthy monkeys on the terrace exposed to the bites of mosquitoes, and one of them, No. 48, was in a cage next to an infected monkey. Yet none of these monkeys have shown the slightest trace of a blood reaction. Direct infection through infected urine seems to be the most probable explanation of the infection. Both Monkey No. 69 and Monkey No. 46 had infected monkeys next to them, and the chance of infection from urine was undoubted, as the *M. melitensis* was discovered in the urine of Monkey No. 46, proving that the specific microbe is excreted from monkeys in the same manner as from human beings. Although the cages and cemented surfaces beneath them were washed with lysol night and morning, still the ground was often noticed covered with decomposing urine.

Having in view the possibility of direct infection from urine excreted by monkeys suffering from Mediterranean fever, it is necessary to enquire whether any of the experiments previously recorded are invalidated by this circumstance. It will be advisable to discuss the experiments *seriatim*.

Experiment I, Monkey No. 41.—This monkey was kept in a small room on the left of the door leading from the laboratory to the roof. It was not placed in its box until infection had been acquired, and even after this it was still separated from Monkey No. 40 by a healthy monkey. It is evident that in relation to this experiment the question of infection by urine could not arise.

Experiment II, Monkey No. 47.—This monkey was placed between two healthy monkeys, viz., No. 46 and No. 48. Monkey No. 48 remained in good health throughout the summer and never showed the slightest sign of infection. Monkey No. 47 was infected on August 8, 1904, but Monkey No. 46 did not show a reaction until September 6, 1904. It is obvious that Monkey No. 47 could not have been infected by urine excreted by its neighbours.

Experiment III, Monkey No. 39.—The monkey was placed between Monkey No. 58 and Monkey No. 66. Monkey No. 58 only received injections of filtered toxins, and could not possibly excrete the specific micrococci in its urine. Monkey No. 66 was directly infected through

a crack in the mouth, and suffered from a marked bacterial infection ; its first rise of temperature occurred on August 10, 1904, and it is practically impossible that the *M. melitensis* could have been excreted in its urine before this date, and, taking into consideration the facts observed in man, it is unlikely that the urine would contain the *M. melitensis* before August 25, 1904. Consequently it seems impossible that the Monkey No. 39 could have received infection from the urine of its neighbours.

Experiment IV, Monkey No. 40.—This monkey was infected on August 11, 1904, and the monkeys nearest to it, viz., 69 and 41, were not infected until September 7, 1904, August 26, 1904, respectively. The question of infection by urine could not arise in this case.

Experiment V, Monkey No. 66.—This monkey was placed between Monkey No. 67 and Monkey No. 39. Monkey No. 67 never showed the slightest trace of infection, and was in good health all the summer. Monkey No. 39, as previously stated, was infected about the same date as Monkey No. 66. It does not seem possible that infection by urine could have played a part in this experiment.

Experiment VI, Monkey No. 72.—This monkey was directly infected through a crack in the mucous membrane of the mouth on September 13 or 16. It was kept apart from infected monkeys.

Experiment VII, Monkey No. 45.—This monkey was directly infected by subcutaneous injection of the *M. melitensis*.

Experiment VIII, Monkey No. 48 } These monkeys failed to become
Experiment IX, Monkey No. 43 } infected.

Experiment X, Monkey No. 46.—This monkey was infected on September 6, 1904, and it appears practically certain that the infection was caused by the specific micrococci present in the urine of neighbouring monkeys.

Experiment XI, Monkey No. 42.—This monkey probably only received toxins contained in the urine excreted by a case of Mediterranean fever.

Experiment XII, Monkey No. 55.—This monkey was directly infected by the subcutaneous injection of the *M. melitensis*.

Experiment XIII, Monkey No. 69.—This monkey became infected on September 7, 1904. The source of infection was probably the urine of its neighbours.

List of Monkeys, not infected, artificially infected, and naturally infected, with Dates of Arrival and Infection.

| No. | Infection. | Arrival. | Remarks. |
|-----|--------------------------------|----------|------------------------------------|
| 70. | Not infected. | 8/8/04. | Dr. Zammit's mosquito experiments. |
| 65. | " | " | |
| 64. | " | " | |
| 63. | Artificially infected. | " | |
| 62. | Not infected. | 16/7/04. | " |
| 61. | " | " | Died, diarrhoea, 26/8/04. |
| 60. | " | " | |
| 59. | " | " | |
| 49. | " | 8/8/04. | Died 11/9/04. |
| 48. | " | 1/7/04. | Died. Experiment II, page 48. |
| 47. | Artificially infected | " | |
| | 9/8/04. | " | |
| 46. | Naturally infected | " | Subcutaneous injection 9/7/04. |
| | 6/9/04. | " | |
| 45. | Artificially infected | " | |
| | 15/7/04. | " | Died from pneumonia, 6/7/04. |
| 44. | Not infected. | " | |
| 43. | " | " | |
| 42. | (?) Infected (probably toxine) | " | Urine infection. |
| 67. | Not infected. | 8/8/04. | Mosquito experiment. |
| 66. | Artificially infected | " | Food experiment. Serum 18/8/04. |
| | ? 9 or 10/8/04. | " | Food experiment. |
| 39. | Artificially infected | 1/7/04. | |
| | 10/8/04. | " | |
| 58. | Not infected. | 16/7/04. | Toxine injected. |
| 57. | " | " | Died from diarrhoea 15/8/04. |
| 56. | " | " | " 5/8/04. |
| 55. | Infected 4/8/04. | " | Culture from urine. Serum 4/8/04. |
| 54. | Not infected. | " | Skin scraping. |
| 68. | " | 8/8/04. | Experiment IV, page 53. |
| 40. | Artificially infected | 1/7/04. | |
| | 11/8/04. | " | |
| 69. | Naturally infected | 8/8/04. | Died. Experiment I, page 46. |
| | 7/9/04. | " | |
| 41. | Artificially infected | 8/7/04. | |
| | 26/8/04. | " | |

MOSQUITO EXPERIMENTS.

These experiments were undertaken in order to ascertain whether the *Stegomyia fasciata* is able to convey the *M. melitensis* from the peripheral blood of Malta Fever patients to healthy monkeys.

Experiment I.

In this experiment the mosquitoes were fed on Private Lawrence, 2nd Essex Regiment. This particular patient was selected, as Staff-Surgeon Shaw had found the maximum number of micrococci in his blood. The number of mosquitoes and the dates on which they were

fed on the patient and on Monkey No. 70, are shown in Table I. An endeavour was made to keep the mosquitoes alive as long as possible, as in view of the work done on Yellow Fever it seemed possible that several days might intervene between the absorption of the *M. melitensis* into the stomach of the mosquito and its transfer, possibly through the salivary glands, to the proboscis. In Dr. Zammit's successful experiment only 48 hours intervened between the absorption of the micrococci and their transfer to the patient. In Experiment I the intervals were 2, 4, 8, and 10 days, respectively. Monkey No. 70 had been under observation for several months and always appeared perfectly healthy. Its serum was examined at varying periods, but it never manifested the slightest power of agglutinating the *M. melitensis*.

Experiment II.

The same procedure was followed in this experiment, the patient, Private K—, R.A.M.C., having a typical wave of fever. The number of mosquitoes and the dates when they were fed on the patient and on Monkey No. 44, are given in Table II. The mosquitoes were kept alive for 13 days, and yet no trace of agglutination could be detected when the serum of the monkey, in a low dilution, was added to an emulsion of the *M. melitensis*.

Experiment III.

In this experiment mosquitoes were fed on different patients, specially selected owing to the presence of marked fever at the time of feeding. The details of the various feedings are given in Table III. All the agglutination tests were negative.

Experiment IV.

In this experiment mosquitoes were fed on monkeys recently inoculated with *M. melitensis* and, after an interval of 48 hours, transferred to Monkey No. 76 which arrived at the laboratory on 8.9.04. On 16.9.04 and 22.9.04 the serum of Monkey 76, diluted 1—10, was added to an emulsion of *M. melitensis*; no agglutination was observed on either occasion. On the 20.9.04 mosquitoes were fed on Monkey No. 60A, at that time at the summit of a wave of fever, and 48 hours later they were fed on Monkey No. 76. On the 25.9.04, mosquitoes were again fed on Monkey No. 60A, and on the 27.9.04 transferred to Monkey No. 76. On the 27.9.04 mosquitoes were fed on Monkey No. 72, infected by feeding and at the height of a wave of fever, and 48 hours later transferred to Monkey No. 76. The serum of Monkey No. 76 was examined on 27.9.04, but did not manifest the slightest power of agglutinating the *M. melitensis*.

(These experiments are still proceeding.)

Table III.—Monkey No. 56. Mosquito Experiments (continued up to the end of October).

| Mosquitoes fed on patients. | | Mosquitoes fed on monkey. | | | | | | | | | | | | | | | | | | | | | |
|-----------------------------|--------------------|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Date. | No. of mosquitoes. | Number of days after being fed on patient. | | | | | | | | | | | | | | | | | | | | | |
| Sept. 4 1904. | 1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
| " 16 | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 22 | 1 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 23 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 25 | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 26 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| Oct. 10 | 6 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 19 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| " 24 | 4 | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

Monkey No. 56 was bitten 104 times by presumably infected mosquitoes. Its serum was repeatedly tested as to agglutination of the *M. melitensis*, but no signs of a reaction were observed.

The want of success, which has up to the present attended our efforts to transfer by means of mosquitoes the *M. melitensis* from infected human beings to healthy monkeys, is disappointing but does not necessarily invalidate the result obtained by Dr. Zammit. The case upon which he made his successful experiment was unusually severe, and since then cases of this type have not been met with either in the military or in the civil hospitals.

Conclusions drawn as to the Mode of Entrance of the M. melitensis into the Body.

There is experimental evidence to show that the *M. melitensis* when present in dry dust is capable of being absorbed by monkeys.

The path of absorption may be through the nares, throat, respiratory passages, and alimentary canal. When present in food it is also taken into the system of monkeys; here, again, the path of absorption may be through the throat as well as through the mucous membrane of the alimentary canal.

When transmitted through an unbroken mucous membrane the process of absorption is comparatively slow, and under these conditions the wave of fever appears to be prolonged. The long and variable incubation period observed in monkeys infected through an unbroken mucous membrane is frequently observed in man infected under natural conditions.

When the *M. melitensis* is absorbed through a crack in a mucous membrane or in the skin, or is injected subcutaneously, the absorption is rapid and the incubation period in monkeys varies from 5 to 7 days. The curve of fever is characterised by a rapid rise usually followed by a rapid fall. These acute infections have also been observed in man infected under the same conditions, but the period of incubation appears to be longer in man than in the monkey.

The history of Monkeys Nos. 69 and 47 shows that healthy monkeys may become infected by urine secreted by monkeys suffering from Mediterranean Fever. Just as in the case of man, the *M. melitensis* is excreted in the urine of infected monkeys. And it seems probable that healthy monkeys walking in the infected secretion convey the specific microbe into the mouth by means of the paws.

Infection by means of urine secreted by cases of Mediterranean Fever readily explains the cases of Mediterranean Fever which appear to arise spontaneously in hospitals. In the absence of specific knowledge as to the mode of excretion of the *M. melitensis* from the human body, sufficient care has hitherto not been taken to sterilise bed-pans, urine bottles and sheets soiled by cases of Mediterranean Fever.

There is no evidence that Mediterranean Fever can be contracted by contact with cutaneous surfaces, uncontaminated by urine.

The experiments made with *Stegomyia fasciata* do not support the result obtained by Dr. Zammit.

5.

DESCRIPTION OF A METHOD OF CULTIVATING THE *MICROCOCCUS MELITENSIS* FROM SMALL QUANTITIES OF PERIPHERAL BLOOD AND INOCULATION EXPERI- MENTS WITH THE MICRO-ORGANISMS ISOLATED.

By Staff-Surgeon R. T. GILMOUR, R.N., Bighi Hospital, Malta.

[*Note*.—This work was kindly undertaken by Staff-Surgeon Gilmour, R.N., at the laboratory of the Naval Hospital, Malta. He has already published a paper on the subject entitled "A few Notes on the Bacteriology and Pathology of Mediterranean Fever," published in 'Health of the Navy' for 1902. In that paper he gives the result of the examination of sixteen cases of Mediterranean Fever. Out of these sixteen cases the *M. melitensis* was isolated from eight, three gave no growth, and five were uncertain as they were contaminated. In these first experiments Staff-Surgeon Gilmour used fairly large quantities of blood and incubated the blood in a large volume of broth. From 0.5—8.8 c.c. blood in from 15—60 c.c. of broth were used.—ED.]

Preparation of the Patient.

The arm should be chosen in which the veins at the bend of the elbow are the most prominent. The selected limb should be shaved from the middle of the arm to the middle of the forearm. This area should then be washed with hot sterile water, carbolic soap, and a sterile nail-brush for 20 minutes; then swabbed with ether for 10 minutes, to dissolve out the fat, and finally scrubbed with a 1 in 500 solution of perchloride of mercury for $\frac{1}{4}$ hour. A sterile dressing should then be applied, soaked in the same disinfectant, until the time of the operation, about 24 hours afterwards.

The Apparatus Required.

1. A sterile bandage.
2. A sterile 10 c.c. serum syringe.

3. (a) One flask, containing 30 c.c. of broth.
 (b) Two tubes, each containing 9 c.c. of broth.*
 (c) Sufficient Petri's dishes, each containing 10 c.c. of agar-agar.
4. A spirit lamp.
5. Sterile 1 c.c. pipettes and glass rods.
6. Six tubes, each containing 10 c.c. of broth.

Method of Extracting the Blood.

1. Remove the bandage from the dressing.
2. Constrict the arm above the elbow-joint with the sterile bandage.
3. After waiting a few minutes, so that the veins may become engorged, insert the needle into the most prominent vein and withdraw sufficient blood, about 5 c.c.

4. An assistant, holding the flask and the tubes on the slant, should then remove the plugs with sterile forceps, and the required quantities of blood (2 c.c. for the flask, and 1 c.c. for the two 9 c.c. tubes) should be passed into the broth.

The assistant should then keep the broth in the tubes well agitated, so as to prevent coagulation and get a good emulsion.

0.5 c.c. of blood should then be passed into each of the Petri dishes, and immediately spread out with a sterile rod.†

5. The next part of the procedure must be performed in the laboratory. Pass the following quantities of emulsion, from one of the 9 c.c. tubes, into others containing 10 c.c. of broth:—‡

| | | | |
|----------------------|---|----------------------|--------------------|
| 0.1 c.c. of emulsion | = | (0.01 c.c. of blood) | into the 1st tube. |
| 0.25 " | " | (0.025 " | " ") " 2nd " |
| 0.5 " | " | (0.05 " | " ") " 3rd " |
| 1.0 " | " | (0.1 " | " ") " 4th " |
| 2.0 " | " | (0.2 " | " ") " 5th " |
| 3.0 " | " | (0.3 " | " ") " 6th " |

6. Incubate the broth tubes and Petri's dishes at 35° C., and examine daily. From the 4th to 10th day of incubation inoculate sloped agar tubes from the broths, allowing 15 drops to flow over the surface of each. Ring all colonies daily, which appear in the Petri dishes, and number them, keeping a tally of the day they appeared. From the 4th to the 10th day remove the colonies with a sterile loop, plant on agar, and incubate at 35° C.

The following are the tests applied to ascertain whether a growth is *M. melitensis*:—

* Tubes containing 19 c.c. of broth were afterwards used.

† These dishes were afterwards inoculated with 1 c.c. of 1—10 emulsion.

‡ Smaller quantities of blood were afterwards used.

1. An emulsion in normal saline is examined under the microscope.
2. Specimens are stained with Neelson's carbol-fuchsin (1 in 10).
3. Specimens are stained by Gram's method.
4. The growth is tested for agglutination with the sera of Mediterranean fever cases; controls being made with healthy serum.

The reaction of all media used in the experiments is + 10A (Eyre's scale) unless otherwise stated.

Experiment I.

Harry Chapman, 28. Admitted into hospital on April 2, 1904. On June 23, 1904, the 84th day of illness, 8.0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|--|
| 4.0 c.c. | 30 c.c. | Pure culture of <i>M. melitensis</i> . |
| 1.0 " | 10 " | " " |
| 1.0 " | 9 " | Used for inoculating the following tubes. |
| 0.01 " | 10 " | Negative. |
| 0.025 " | 10 " | " |
| 0.5 " | 10 " | " |
| 0.1 " | 10 " | " |
| 0.2 " | 10 " | " |
| 0.3 " | 10 " | " |

Result of Inoculations of Blood on to Sloped Agar Tubes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|--|
| 0.5 c.c. | 10 c.c. | One colony of <i>M. melitensis</i> . |
| " | " | Three colonies of <i>M. melitensis</i> . |
| " | " | Sterile. |

Experiment II.

J. S. Ward, 24. Admitted into hospital on June 8, 1904. On June 25, 1904, the 17th day of illness, 5.0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|------------------------------------|
| 2·0 c.c. | 30 c.c. | Contaminated. |
| 1·0 „ | 9 „ | The <i>M. melitensis</i> obtained. |
| 0·01 „ | 10 „ | Sterile. |
| 0·025 „ | 10 „ | „ |
| 0·05 „ | 10 „ | „ |
| 0·1 „ | 10 „ | „ |
| 0·2 „ | 10 „ | The <i>M. melitensis</i> obtained. |
| 0·3 „ | 10 „ | Sterile. |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---|
| 0·5 c.c. | 10 c.c. | Five colonies of <i>M. melitensis</i> obtained. |
| 0·25 „ | 10 „ | Contaminated. |

Experiment III.

Alfred Law, 20. Admitted into hospital on June 20, 1904. On June 28, 1904, the 14th day of illness, 5·0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|--------------------------------|
| 2·0 c.c. | 30 c.c. | Contaminated. |
| 0·01 „ | 10 „ | Sterile. |
| 0·025 „ | 10 „ | <i>M. melitensis</i> obtained. |
| 0·5 „ | 10 „ | „ „ |
| 0·1 „ | 10 „ | „ „ |
| 0·2 „ | 10 „ | „ „ |
| 0·3 „ | 10 „ | „ „ |
| 0·01 „ | 10 „ | „ „ |
| 0·025 „ | 10 „ | „ „ |
| 0·05 „ | 10 „ | „ „ |
| 0·1 „ | 10 „ | „ „ |
| 0·2 „ | 10 „ | Broth contaminated. |
| 0·3 „ | 10 „ | „ „ |

Experiment IV.

John Waters, 23. Admitted into hospital on June 29, 1904. On July 5, 1904, the 25th day of illness, 8·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|------------------------------------|
| 2.0 c.c. | 30 c.c. | The <i>M. melitensis</i> obtained. |
| 1.0 " | 9 " | Sterile. |
| 0.01 " | 10 " | " |
| 0.025 " | 10 " | " |
| 0.05 " | 10 " | " |
| 0.1 " | 10 " | " |
| 0.2 " | 10 " | " |
| 0.3 " | 10 " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|--|
| 0.5 c.c. | 10 c.c. | One colony of <i>M. melitensis</i> . |
| " | " | " " |
| " | " | Contaminated. |
| " | " | Two small contaminations. No <i>M. melitensis</i> . |
| " | " | One small contamination. No <i>M. melitensis</i> . |

Experiment V.

Thomas Eccles, 23. Admitted into hospital on July 9, 1904. On July 19, 1904, the 23rd day of illness, 2.0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes,

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|---------------|
| 0.01 c.c. | 10 c.c. | Contaminated. |
| 0.025 " | " | " |
| 0.05 " | " | " |
| 0.2 " | " | " |
| 0.2 " | " | " |

Result of Inoculations of Blood on to Sloped Agar Tubes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---------------|
| 0.1 c.c. | 10 c.c. | Contaminated. |
| " | " | " |
| " | " | " |
| " | " | " |
| " | " | " |

The whole of these growths were contaminated with a staphylococcus.

Experiment VI.

Edward Stedman, 32. Admitted into hospital on July 7, 1904. On July 20, 1904, the 25th day of illness, 3·5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|-----------------------|------------------------|--------------------------------------|
| 0·1 c.c. | 10 c.c. | One colony of <i>M. melitensis</i> . |
| " | " | Sterile. |
| " | " | Contaminated. |
| " | " | Sterile. |

Experiment VII.

Sidney Fleetwood, 23. Admitted into hospital on June 11, 1904. On July 21, 1904, the 40th day of illness, 4·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|-----------------------|------------------------------|--|
| 1·0 c.c. | 50 c.c. | Pure culture of <i>M. melitensis</i> . |
| 0·01 " | 10 " | Sterile. |
| 0·025 " | 10 " | " |
| 0·05 " | 10 " | " |
| 0·1 " | 10 " | " |
| 0·2 " | 10 " | " |
| 0·3 " | 10 " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|-----------------------|------------------------|--------------------------------------|
| 0·1 c.c. | 10 c.c. | Sterile. |
| " | " | " |
| " | " | " |
| " | " | " |
| " | " | One colony of <i>M. melitensis</i> . |

Experiment VIII.

James Slater, 21. Admitted into hospital on July 13, 1904. On July 22, 1904, the 20th day of illness, 3·5 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|--|
| 1.5 c.c. | 30 c.c. | Pure culture of <i>M. melitensis</i> . |
| 0.005 " | 10 " | Sterile. |
| 0.0125 " | 10 " | " |
| 0.025 " | 10 " | " |
| 0.05 " | 10 " | " |
| 0.1 " | 10 " | " |
| 0.15 " | 10 " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---------------|
| 0.1 c.c. | 10 c.c. | Sterile. |
| " | " | Contaminated. |
| " | " | Sterile. |
| " | " | Contaminated. |
| " | " | " |
| " | " | Sterile. |

Experiment IX.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 12, 1904, the 3rd day of illness, 3.5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|---|
| 1.0 c.c. | 30 c.c. | Pure culture of <i>M. melitensis</i> obtained. |
| 0.005 " | 10 " | Sterile. |
| 0.0125 " | 10 " | " |
| 0.025 " | 10 " | " |
| 0.05 " | 10 " | " |
| 0.1 " | 10 " | " |
| 0.15 " | 10 " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---|
| 0·1 c.c. | 10 c.c. | One colony of <i>M. melitensis</i> , and one small colony of contamina- tion. |
| " | " | One small colony of contamina- tion. |
| " | " | Sterile. |
| " | " | Contaminated. |
| " | " | " |
| " | " | " |

Experiment X.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 19, 1904, the 10th day of illness, 2·5 c.c. of blood were with-
drawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|--------------------------|---------------------------------|---------------|
| 0·005 c.c. | 10 c.c. | Sterile. |
| 0·0125 " | " | Contaminated. |
| 0·025 " | " | " |
| 0·05 " | " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---|
| 0·1 c.c. | 10 c.c. | Sterile. |
| " | " | One small colony of contamina- tion. |
| " | " | Contaminated. |

Experiment XI.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 19, 1904, the 5th day of illness, 1·0 c.c. of blood was withdrawn
from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|--------------------------|---------------------------|---------------------------------------|
| 0·1 c.c. | 10 c.c. | 31 colonies of <i>M. melitensis</i> . |
| " | " | 33 " " |
| " | " | 31 " " |

Experiment XII.

Edward Freak, 21. Admitted into hospital on August 18, 1904. On August 22, 1904, the 24th day of illness, 1.0 c.c. of blood was withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|-----------------------|------------------------------|---------------|
| 0.005 c.c. | 10 c.c. | Sterile. |
| 0.0125 " | " | " |
| 0.025 " | " | Contaminated. |
| 0.05 " | " | Sterile. |
| 0.1 " | " | " |

Experiment XIII.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 27, 1904, the 13th day of illness, 3.0 c.c. of blood were withdrawn from the right median-cephalic vein.

Result of Inoculations of Blood into Broth Tubes.

| Amount of blood used. | Amount of medium broth used. | Result. |
|-----------------------|------------------------------|----------|
| 0.0025 c.c. | 10 c.c. | Sterile. |
| 0.005 " | " | " |
| 0.0125 " | " | " |
| 0.025 " | " | " |
| 0.05 " | " | " |

Result of Inoculations of Blood on to Petri's dishes.

| Amount of blood used. | Amount of medium used. | Result. |
|-----------------------|------------------------|---------------|
| 0.1 c.c. | 10 c.c. | Sterile. |
| " | " | " |
| " | " | Contaminated. |

On August 19, 1904, this man's blood had given 316 micrococci per cubic centimetre, *vide* Experiment XI.

Table showing the Average Number of *M. melitensis* per cubic centimetres of Blood and the Day of Disease.

| Experiment. | Day of disease. | Number of micrococci per cubic centimetres of blood. |
|-------------|--------------------|---|
| I | 84 | 2·6 |
| II | 17 | 10 |
| III | 14 | 100 |
| IV | 25 | 1·0 |
| V | 23 | 0·0 |
| VI | 25 | 3·3 |
| VII | 40 | 2·0 |
| VIII | 20 | 0·6 |
| IX | 3 | 3·3 |
| X | 10 | 0·0 |
| XI | 5 | 316·6 |
| XII | 24 | 0·0 |
| XIII | 13 | 0·0 |

[*Remarks.*—It is evident from Staff-Surgeon Gilmour's experiments that the *M. melitensis* is present in the majority of the cases examined. Their number is, however, so small that it seems extremely doubtful if this disease can be carried by biting insects.—ED.]

INOCULATION EXPERIMENTS ON MONKEYS WITH MICRO-ORGANISMS, SUPPOSED TO BE *M. melitensis*, FROM THE BLOOD OF PATIENTS, SUFFERING FROM MEDITERRANEAN FEVER.

Experiment I.

A small, healthy, female Rangoon monkey, which had been under observation for 20 days. It had gone up in weight $\frac{1}{2}$ lb., its coat had improved, and it appeared in perfect health. The temperature varied between 99°·6 and 101°·8. Its serum did not agglutinate *M. melitensis* in a dilution of 1—10. Weight 4 lbs. 12 ozs.

The object of this experiment was to prove that the coccus, obtained from the peripheral blood of a patient (W. A., age 32), was the *M. melitensis*.

October 6, 1903. This monkey was inoculated between the shoulder blades with an emulsion made from the contents of two sloped agar tubes (third generation of micrococcus) in 1 c.c. of broth.

October 7, 1903. Weight 4 lbs. 12 ozs.; appears well.

October 8, 1903. Weight 4 lbs. 10 ozs.; eating well.

October 9, 1903. Weight 4 lbs. 10 ozs.; seedy.

October 11, 1903. Weight 4 lbs. 5 ozs.; irritable, in other respects appears well. Its serum gives an immediate reaction to *M. melitensis* 1—10, 1—50, and 1—100 after 24 hours,

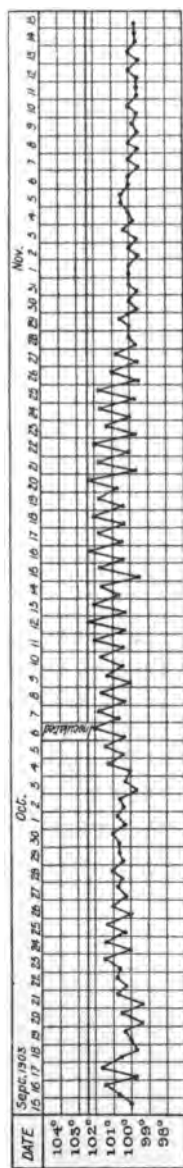
October 15, 1903. Weight 4 lbs. 2 ozs.; good reaction 1—100; seedy, but not very ill.

October 20, 1903. The monkey is improving in health. Slight reaction 1—50; good reaction, 1—30.

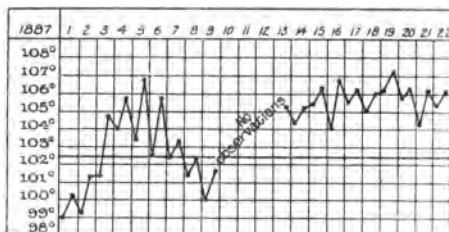
November 1, 1903. The animal has regained its weight and now weighs 4 lbs. 12 ozs. Perfectly well; reaction 1—10.

June 10, 1904. This monkey still reacts 1—10. It had no relapse.

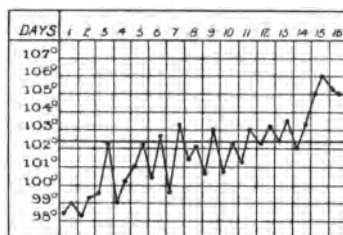
The following chart represents the temperature curve. Taken in the axilla.



[*Remarks.*—The temperature seems to have been taken in the axilla. It ought, in my opinion, to be taken in the rectum, the thermometer should be introduced as far into the intestine as possible, and a minimum of 5 minutes used for the observation. It is difficult to believe that this monkey can have had Malta Fever. The temperature chart shows no signs of the disease. Compare the following charts :—

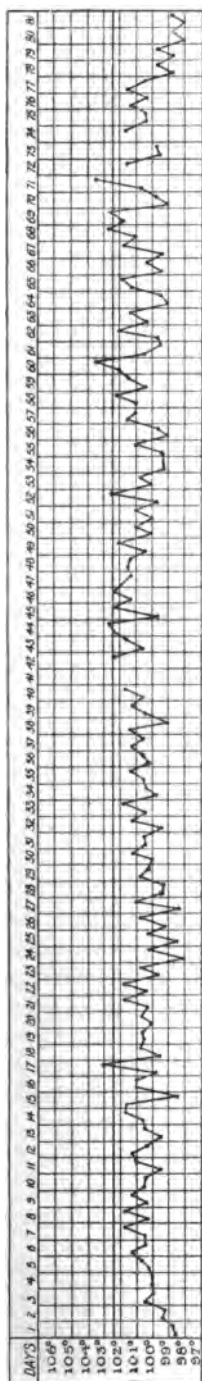


Monkey ♂. *Macacus rhesus*. Bruce. Temperature taken in the Axilla.
Growth from Spleen.

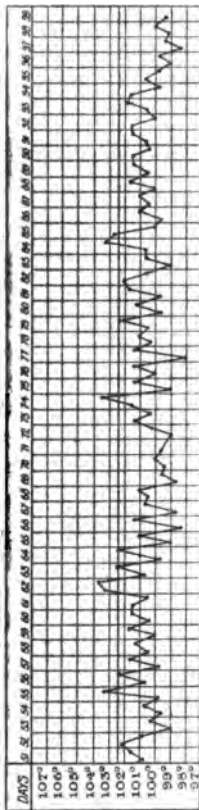
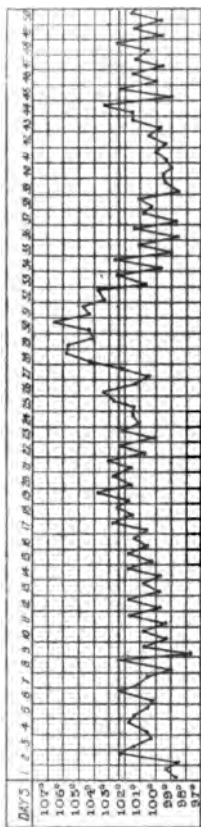


Monkey ♂. *M. rhesus*. Hughes. Axilla Temperature. Growth from Spleen.

Compare also the charts, Experiments V, VI, and XI Horrocks. In these cases the *M. melitensis* was recovered from the spleen after death. All these charts show a definite febrile disturbance, which is almost absent in the chart under consideration. It is certainly desirable that in these cases, the animal should be killed and the *M. melitensis* looked for in the spleen. Of course there is always the danger that the taking of the animal's temperature is entrusted to an ignorant or untrustworthy assistant.—ED.]



Monkey δ. Hughes. Axilla. Growth from Heart's Blood of Monkey.



Monkey q. M. rhesus. Hughes. Axilla. Growth from Spleen of Monkey.

Experiment II.

A small, healthy, male monkey, which had been kept under observation for 28 days. Weight 4 lbs. 9 ozs. No reaction 1—10.

November 16, 1903. This monkey was inoculated into the extensor muscles of the left thigh with 1 c.c. of an emulsion, made from three tubes of *M. melitensis* (first generation) in 2 c.c. of broth.

This experiment was carried out to prove that the growth, obtained from the peripheral blood of G. F., was the *M. melitensis*.

November 24, 1903. The monkey appears perfectly well. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400.

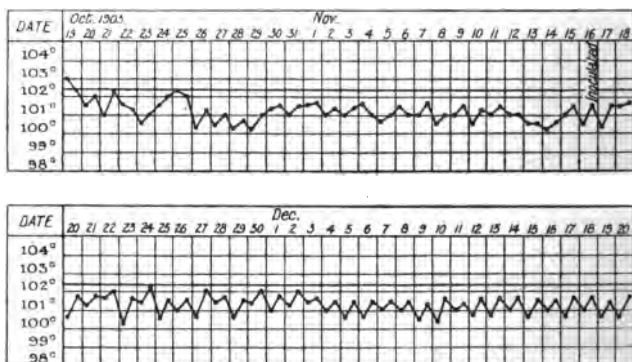
November 27, 1903. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400. The monkey was given a second injection of the contents of one tube, from same patient, into the muscles of the right thigh.

December 8, 1903. The monkey has remained perfectly well. Weight 4 lbs. 8 ozs. Agglutination reaction 1—200.

January 3, 1904. Monkey in good health. Agglutination reaction 1—200.

June 10, 1904. This monkey still reacts 1—10.

The following chart represents the temperature, taken in the axilla :



[Remarks.—This is also a very unsatisfactory temperature chart. The high agglutination reaction is, however, a strong argument that Staff-Surgeon Gilmour is dealing with *M. melitensis*.—ED.]

Experiment III.

The following experiment was carried out to prove that the coccus, obtained from the knee-joint of F. B., age 21, was the *M. melitensis*.

December 5, 1903. A male monkey, which had been under observation for a week, was inoculated into the extensor muscles of the left

thigh with an emulsion made from one tube (fourth generation) in 1 c.c. of sterile broth. It weighed 7 lbs. 6 ozs.; its serum would not agglutinate the laboratory *M. melitensis*; and its temperature was steady, 100° F.—100°·6 F.

The monkey remained well until December 8, the 3rd day after inoculation, when it shivered a good deal, went off its feed, and suffered from a rise of temperature, 102° F., in the evening.

After this date the monkey became very sick; its serum gave a negative reaction 1—10 on December 8; reacted 1—1200 on December 13, 1—1200 on the 17, and 1—3000 on the 20, the 15th day after inoculation; its weight decreased 1 lb. 4 ozs. by December 22, and its temperature remained up after the 3rd day, ranging between 101° and 102°·8 F.

December 23, 1903. The monkey was killed with chloroform, and a *post-mortem* held.

The organs were healthy, with the exception of the liver and spleen, which were congested. There were no signs of tubercle. Two sloped agar and two broth tubes were inoculated from the liver, three agar and two broth from the spleen, two agar from the heart's blood, and 30 c.c. of broth with 1 c.c. of heart's blood.

December 29, 1903. The tubes from the liver remained sterile and were destroyed.

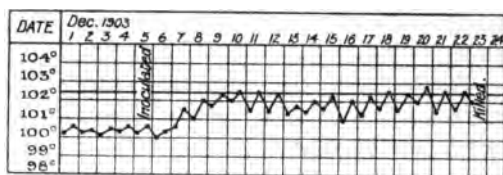
The agar tubes from the spleen showed no growth until the 3rd day, December 26, when many small isolated colonies appeared which, by the 4th day, had the appearance of a growth of *M. melitensis*. One broth tube from the same organ gave a growth by the 5th day; the other was sterile. A few transparent, isolated colonies also appeared on one agar tube from heart's blood on the 4th day.

The broth inoculated with heart's blood remained clear until the 3rd day, when it became slightly cloudy, after which the coccus grew rapidly; each field under the microscope being full of cocci. Sloped agar tubes (1 and 2), inoculated from the blood broth on December 24 and 27 respectively, remained sterile and were destroyed on December 29 and January 2. Two other tubes (3 and 4), inoculated on December 28, showed growth on December 31—isolated, transparent colonies, which the next day had every appearance of *M. melitensis*.

The tubes from the blood and spleen were examined microscopically, etc., and the growth—a micrococcus—was found to be identical in size, shape, motility, and staining reactions with the laboratory *M. melitensis*; it also gave an immediate agglutination reaction with the sera of the following Mediterranean fever patients: A. 1—500, B. 1—1000, P. 1—100, but not with healthy serum.

I think that the above experiments prove conclusively that the coccus, obtained in the first place from the synovial fluid of the knee-joint, was the *M. melitensis*.

The following chart shows the temperature curve :



[Remarks.—There can be little doubt that in this case Staff-Surgeon Gilmour is dealing with the *M. melitensis*. There is a distinct rise of temperature and the micro-organism was recovered from the spleen and blood.—ED.]

6.

ISOLATION OF THE *MICROCOCCUS MELITENSIS* FROM THE BLOOD.

By Dr. T. ZAMMIT, Member Mediterranean Fever Commission.

The patients of the Civil Central Hospital furnished, for the most part, the material for this investigation. The Honourable the Comptroller of the Charitable Institutions and the Medical Officers connected with that hospital deserve the thanks of the Commission for having kindly allowed the investigation to be conducted in the hospital.

The method followed at first was the simple one of drawing blood with a syringe from a vein at the bend of the arm. One to five cubic centimetres of blood was drawn, with all necessary precautions, and diluted in broth in the proportion of 1 of blood to 19 of broth. A proportion of 1 to 9 of broth was tried, but found unsuitable.

As soon as the blood was mixed with the broth it was taken to the laboratory where it was put in various proportions in 10 c.c. broth tubes and incubated. From the first mixture of blood 1, 2, 3, 4, 5 c.c., etc., were added to broth tubes and the dilution noted.

After an incubation of 4—5 days, a loopful of broth was passed over a sloped agar tube. When after 5 days no growth appeared on the agar, the same tube was reinoculated from the corresponding tube of broth, and so on every 5 days up to 1 month.

If a growth appeared having the appearance of the *M. melitensis* a note was made and the tube set aside for identification; if numerous

foreign growths appeared, the tube was usually thrown away and a note made that it was contaminated.

In some cases, however, the *M. melitensis* could be easily recognised among a lot of contaminations, and then sub-cultures were made to get a pure culture of the Micrococcus.

The contaminations observed during this investigation were traced to the imperfect preparation of the skin before drawing the blood, and, in fact, the contaminations were reduced to a minimum when a pad with carbolic solution (5 per cent.) was kept on the part for a few hours previous to the operation.

No bad effects were ever observed after the puncture, and no complaints were ever made by the patients.

After some time a few cases were met with in which, owing either to the prostrate condition of the patient or to his excessive nervousness the drawing of the blood from a vein by means of a syringe was not found to be possible. I, therefore, devised the following method of taking the blood which has proved so successful that I resorted to it constantly afterwards:—

The finger or the lobe of the ear of the patient is washed well with ether, ether-soap, water, alcohol and ether, and on the dry skin a puncture is made with a small syringe needle. With a sterile cotton wool pad the first drop of blood is removed, and an assistant squeezes the part for the next drop at the request of the operator. In a test-tube a large number of capillary tubes 1 cm. long are sterilised by dry heat, and at the time of collecting the blood, one of these short tubes is taken with fine forceps passed, immediately before, through the flame. As soon as the assistant squeezes the part and removes the cotton-wool pad, the tube is brought in contact with the drop, and when full is immediately put in a broth tube. This operation is repeated as long as the blood continues to ooze; six tubes are usually filled. From these broth tubes, marked and incubated, passages on agar are made in the usual manner.

When a growth of *M. melitensis* is obtained on the agar slope, the capillary tube is drawn out of the broth, washed, dried, and weighed. It is then weighed again full of distilled water, and the difference between the two weights gives the volume of liquid the tube can hold, thus establishing to a nicety the amount of blood from which the *M. melitensis* has been isolated. By this method a volume of 0.005 of 1 c.c. of blood has been easily and accurately measured.

This method was used in twenty-two cases out of fifty with good results. Greater care is, of course, required in the disinfection of the skin, but when this extra trouble is taken the results compare most favourably with the bleeding from a vein. This method has also the great advantage that it can be applied to animals, as in case No. 50 in Table A.

Table A.

| Order number. | Name and surname. | Sex. | Age. | Date of illness. | Character of case. | Temperature of body at time of experiment. | Amount of blood taken. | Minimum amount of blood in which <i>M. melitensis</i> was found. | Date of observation. | Remarks. |
|---------------|---------------------------|------|------|------------------|--------------------|--|------------------------|--|----------------------|---------------------|
| 1 | Giorgio Abdilla | M | 40 | Day. | Mild | 99.8 | c.c. | c.c. | 1904. | No growth whatever. |
| 2 | Paolo Spiteri | M | 49 | 100 | " | 99.0 | 1 | — | June 21 | " |
| 3 | Emmanuele Caruana | M | 24 | 65 | " | 99.8 | 1 | 0.1 | June 23 | " |
| 4 | Ursola Vassallo | F | 56 | 20 | " | 101.0 | 1 | 0.1 | " | " |
| 5 | Salvatore Camilleri | M | 28 | 120 | " | 100.0 | 1 | — | June 27 | " |
| 6 | Maria Chelcuti | F | 29 | 13 | Acute | 100.2 | 1 | 0.2 | " | " |
| 7 | Carmela Dimech | F | 18 | 35 | Mild | 101.0 | 1 | 0.1 | July 7 | " |
| 8 | Giuseppe Cordina | M | 31 | 240 | " | 99.8 | 1 | — | " | " |
| 9 | Alfredo Scicluna | M | 38 | 14 | Acute | 102.0 | 1 | 0.1 | July 8 | " |
| 10 | Pasquale Cachia | M | 33 | 8 | Mild | 99.0 | 1 | — | " | " |
| 11 | Francesco Saliba | M | 45 | 15 | Acute | 100.0 | 1 | 0.1 | July 11 | " |
| 12 | Salvatore Ungaro | M | 25 | 30 | Mild | 99.1 | 1 | — | " | " |
| 13 | Antonia Hili | F | 23 | 8 | Acute | 104.4 | few drops | — | July 15 | " |
| 14 | Luigia Brina | F | 45 | 13 | " | 103.2 | " | 0.02 | " | Tubes contaminated. |
| 15 | | M | 21 | 30 | Mild | 103.2 | 1 | — | " | No growth whatever. |
| 16 | Giuseppe Farrugia | M | 36 | 14 | " | 101.0 | 1 | — | " | " |
| 17 | Mosè Azopardi | M | 29 | 10 | Acute | 103.4 | 1 | 0.1 | July 18 | " |
| 18 | Luigia Brina | F | 45 | 16 | " | 102.0 | 1 | 0.1 | " | " |
| 19 | Raffaele Mercieca | M | 15 | 7 | " | 105.4 | 1 | 0.1 | July 22 | Tubes contaminated. |
| 20 | Carmelo Fava | M | 24 | 13 | Mild | 102.4 | 1 | — | " | " |
| 21 | Simeone Cumbo | M | 30 | 15 | " | 101.4 | 1 | 0.2 | " | " |
| 22 | Giuseppe Micallef | M | 39 | 18 | " | 102.4 | 1 | 0.1 | " | " |
| 23 | Caterina Pons | F | 44 | 54 | " | 102.0 | 1 | 0.1 | July 27 | " |

| | | | | | | | | | | | |
|----|---------------------|---|----|-----|-------|-------|-----------|--------|---------|---------------------|--|
| 24 | Angelo Inguanez | M | 37 | 12 | Acute | 102.0 | 1 | 0.5 | " | | |
| 25 | Nattar Bassar | M | 22 | 7 | " | 101.0 | 1 | 0.1 | " | | |
| 26 | Marianne Grina | F | 24 | 32 | Mild | 101.0 | 1 | 0.1 | " | | |
| 27 | Vincenzo Mamò | M | 27 | 22 | " | 101.0 | few drops | — | " | | |
| 28 | Salvatore Bonanno | M | 46 | 13 | " | 101.0 | " | — | " | | |
| 29 | Carmelo Vella | M | 26 | 150 | " | 99.0 | " | — | " | | |
| 30 | Patrick Bourke | M | 33 | 65 | " | 99.4 | " | — | " | | |
| 31 | Giovanni Buhagiar | M | 29 | 6 | Acute | 102.0 | " | — | Aug. 4 | | |
| 32 | Carmelo Micallef | M | 15 | 33 | Mild | 99.0 | " | 0.0097 | Aug. 9 | | |
| 33 | Giuseppe Zammit | F | 17 | 7 | Acute | 104.0 | " | 0.1 | Aug. 10 | | |
| 34 | Gio. Maria Mifsud | M | 55 | 10 | " | 101.0 | 1 | 0.025 | Aug. 11 | No growth whatever. | |
| 35 | Carmela Zammit | F | 17 | 8 | " | 103.8 | 1 | — | Aug. 11 | " | |
| 36 | Maria Teresa Perini | F | 22 | 21 | Mild | 102.0 | few drops | — | Aug. 12 | " | |
| 37 | Maria Anna Fenech | F | 25 | 30 | " | 102.0 | " | — | Aug. 12 | " | |
| 38 | Nicola Farrugia | M | 48 | 18 | Acute | 100.6 | 1 | 0.025 | " | | |
| 39 | Tancredi Piacentini | M | 31 | 7 | Mild | 102.2 | 1 | 0.05 | " | | |
| 40 | Carmelo Grech | M | 43 | 35 | Acute | 102.4 | 1 | 0.005 | Aug. 17 | | |
| 41 | S. Valder | F | 25 | 7 | " | 104.0 | few drops | 0.009 | " | | |
| 42 | Carmela Bugeja | F | 27 | 17 | " | 105.0 | " | 0.008 | " | | |
| 43 | Giuseppa Grina | F | 43 | 60 | Mild | 106.0 | " | — | Aug. 22 | Tubes contaminated. | |
| 44 | Anna Zammit | F | 40 | 120 | Acute | 103.0 | " | — | Aug. 24 | " | |
| 45 | Jos. Sullivan | M | 6 | 14 | Mild | 102.4 | " | — | Aug. 25 | No growth whatever. | |
| 46 | Carmelo Delicata | M | 56 | 18 | " | 99.0 | " | — | " | | |
| 47 | Vincenzo Abela | M | 32 | 8 | Acute | 101.0 | " | 0.006 | " | | |
| 48 | Gaetano Billion | M | 21 | 6 | Mild | 103.0 | " | — | " | | |
| 49 | Nicola Farrugia | M | 48 | 31 | Acute | 103.0 | " | 0.005 | Aug. 27 | " | |
| 50 | Monkey No. 63 | M | — | 16 | Acute | 105.0 | " | — | " | | |

The examination of fifty cases, made between June 21 and August 27, show that the *M. melitensis* circulates freely in the blood during an attack of fever, and that the amount of Micrococci varies usually with the temperature of the body.

In the fifty cases tabulated the *M. melitensis* was never recovered when the body temperature was below 100° F. At 102° and over it was recovered with the exception of two cases (Nos. 36 and 37), in which the tubes remained sterile, and in four cases in which the tubes were hopelessly contaminated. From one of these cases (No. 15) the *M. melitensis* was isolated by one of my colleagues on the same day.

Attempt to infect a monkey by means of a mosquito which had previously fed on a Mediterranean Fever patient.

Several mosquitoes (*Stegomyia fasciata*), which had previously been fed on an infected monkey (No. 45), were made to bite two healthy monkeys. No positive results were obtained. A positive result was obtained on the third attempt.

The third monkey (No. 63) was bought in Malta, along with two others, from a ship coming from the East Indies. Its temperature was taken twice daily after July 18, and it kept always within normal limits up to August 15.

The monkey was kept on the terrace on a side facing south-east, along with seven other animals, none of which had ever been ill.

On July 27 the blood of this monkey was tested, and it did not react to *M. melitensis* when diluted to 1 in 10.

On August 10 at 11 A.M. the monkey was bitten by two *Stegomyias* which had been fed at 11 A.M. on August 8 on a patient affected with a sharp relapse of Mediterranean Fever at the Civil Hospital (patient P. Sillato, Bed No. 40).

On August 20 the monkey was bitten again by one of the two *Stegomyias* used on the 10th.

On August 23 (13 days after inoculation) a rise of temperature was observed, and the blood of the animal was tested for Mediterranean Fever reaction, but no clear reaction could be obtained.

On August 26 the temperature rose again, and on the blood being tested, it was observed that it reacted strongly to *M. melitensis*. An immediate and complete agglutination was obtained at various dilutions up to 1 in 300. No further dilutions were tried.

The animal had obviously a sharp attack of fever, but the isolation of the coccus from the blood was necessary to make sure of the disease.

Without killing the animal, on August 31 one of its ears was properly disinfected and blood was drawn by pricking a small vein. The

blood was collected in small capillary pipettes 1 cm. long, in the manner described in another part of the Report, and put in broth.

On September 1 passages on agar were made from the broth tubes, and on the 4th a distinct growth was observed in one of the tubes. On the 5th two other tubes were found to have grown the Coccus.

All the growths tested in the ordinary way showed that the microbe was the *M. melitensis* in pure culture.

The least amount of blood from which the *M. melitensis* was obtained in this case was 0.005 c.c. Smaller quantities were not tried.

The position of the other monkeys, both healthy and ill, at the time of the experiment, is shown in the plan (p. 42). It is easily seen that no infected monkeys were anywhere near No. 63, and, therefore, direct infection from the monkeys, then ill on the same terrace, is highly improbable.

EXPERIMENTS MADE IN MALTA BY DR. ZAMMIT BEFORE THE APPOINTMENT OF THE COMMISSION.

1. To Test Vitality of *M. melitensis* on Filter-paper exposed to Diffused Light.

| | | | | | |
|--------------|-----------|--|---|---------------------|---|
| August | 27, 1903. | A strip of filter-paper was hung on a wire inside a test-tube plugged with cotton-wool and sterilised by dry heat. | | | |
| „ | 28, „ | Strip of filter-paper smeared with loopful of agar culture. Twelve tubes prepared in the same manner. | | | |
| September 1, | „ | The filter-paper dropped in a broth tube and incubated. Growth obtained in due time. | | | |
| „ | 2, „ | „ | „ | Same result. | |
| „ | 3, „ | „ | „ | „ | |
| „ | 4, „ | „ | „ | No growth obtained. | |
| „ | 5, „ | „ | „ | „ | „ |
| „ | 6, „ | „ | „ | „ | „ |

Conclusion.—*M. melitensis* retained its vitality for 7 days in diffused light. This experiment was repeated three times with the same result.

2. To Test Vitality of *M. melitensis* in various Coloured Lights.

Agar tubes inoculated with a drop of broth culture were incubated in cardboard boxes, of which the cover was made of a coloured glass plate. Violet, red, green, yellow, and blue plates were used. One tube was left in diffused light, and another one was wrapped in black paper.

Result.—No difference in growth was observed in the different tubes. The experiment was repeated three times with the same result, the tube exposed to blue light showing once a richer growth than the rest.

3. Action of Direct Sunlight on Growth of *M. melitensis* in Agar Tubes.

September 17, 1903. Agar tube inoculated with 1 drop of broth culture was exposed for 15 minutes to the direct action of sunlight at about noon. Control tubes left in diffused light. No growth appeared before the 3rd day, but on the 4th day a growth was seen which in a few days was much more luxuriant than that on control tubes.

The experiment was repeated twice with the same result.

4. Vitality of *M. melitensis* on Ordinary Limestone.

September 12, 1903. Small bits of ordinary white porous limestone were taken and thoroughly sterilised. Emulsion made of *M. melitensis* from agar in sterile distilled water and the bits of stone wetted with this. The whole was kept in a dry atmosphere. On the 3rd day bits of the stone were dropped in broth tubes.

As former experiments had shown that light favours the growth of the *M. melitensis*, part of the bits of stone wetted with *M. melitensis* emulsion was kept in diffused light and part in a tube wrapped in thick black paper. The other conditions of the two tubes with pieces of stone were the same.

The result of the experiment was as follows :—

| | Stone kept in dark. | Stone kept in diffused light. |
|---------------------|----------------------------------|----------------------------------|
| Sept. 15 (3rd day). | Growth of <i>M. melitensis</i> . | Growth of <i>M. melitensis</i> . |
| „ 18 (6th „). | „ „ | „ „ |
| „ 19 (7th „). | „ „ | „ „ |
| „ 20 (8th „). | „ „ | „ „ |
| „ 26 (14th „). | No growth. | „ „ |
| Oct. 28 (46th „). | „ | „ „ |
| Nov. 2 (51st „). | „ | „ „ |
| „ 19 (68th „). | „ | No growth. |

Conclusion.—Vitality of *M. melitensis* on limestone, in the dark, from 8 to 14 days.

Vitality of *M. melitensis* on limestone, in diffused light, not less than 51 days.

The experiment was repeated three times with practically the same result.

5. To Test the Action of *M. melitensis* on the Reaction of Media.

September 22, 1903. Seventy cubic centimetres of peptone broth with a reaction of + 6, Eyre's scale, inoculated with loopful of *M. melitensis* from agar, and incubated at 37° C.

„ 26, „ Acidity reduced to + 2.
October 28, „ Broth distinctly alkaline.

6. October 29, 1903. A series of test-tubes with 20 c.c. of broth in each were inoculated with a loopful of agar culture of *M. melitensis*. The tubes were then placed in large Buchner tubes half full with water and lightly covered so as to reduce the evaporation to a minimum. The whole was then incubated at 37° C. Tubes with broth were put for control in the same conditions.

November 19, „ (20th day). Acidity of broth + 2.

January 21, 1904 (82nd „). Broth alkaline - 3.

February 18, „ (110th „). „ - 4.5.

The control tubes showed an increased acidity. On the 20th day the acidity in the control tubes had doubled.

(This experiment is being repeated.)

7.

INTERIM REPORT OF EXPERIMENTAL WORK IN THE INVESTIGATION OF MEDITERRANEAN FEVER DEALING WITH BLOOD, SKIN, SWEAT, FILTRATIONS, AGGLUTINATING SERUM AND VARIOUS INOCULATIONS ON DIFFERENT ANIMALS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission.

Examination of Blood.

The peripheral blood of Malta Fever patients has been examined by me for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*) in fifty-one cases, the results of which I append in a tabular form.

Method.—Bend of elbow prepared as for a surgical operation, blood withdrawn from median-basilic vein direct by means of carefully sterilised serum syringe.

| | | | | |
|--|---|---------------------------------------|-----------------|------|
| $\frac{1}{2}$ c.c. distributed over surface of agar in a Petri dish A. | | | | |
| 1 | „ | „ | „ | B. |
| 2 | „ | „ | „ | C. |
| 1 | „ | put into a 19 c.c. peptone broth tube | | „ D. |
| 1 | „ | „ | another 19 c.c. | „ E. |

ABCD kept intact, E used for making dilutions immediately, first well mixing blood and broth through a series of broth tubes by means of graduated pipettes sterilised in boiling water. At first the dilutions proceeded by multiples of 10; for instance, tube D contained 1 c.c. blood and 19 c.c. broth = a dilution of $\frac{1}{20}$, $2\frac{1}{2}$ c.c. of this contained $\frac{1}{8}$ c.c. blood and added to a 10 c.c. broth tube = $\frac{1}{8}$ c.c. of blood in $12\frac{1}{2}$ of mixture = a dilution of $\frac{1}{100}$; and abstracting 1 c.c. of this ($\frac{1}{100}$ c.c. of blood) and adding to a 9 c.c. broth tube = $\frac{1}{100}$ c.c. of blood in 10 of mixture = $\frac{1}{1000}$ dilution and so on up to $\frac{1}{100000}$.

All broth tubes and plates were duly labelled with a serial number for each patient, the quantity of blood contained, and the date and placed in the incubator at 37° C.

As time went on and the series of bloods increased it was found that *M. melitensis* was only being recovered from relatively large quantities of blood, up to Blood 15 never even from $\frac{1}{100}$ c.c. of blood and only occasionally from $\frac{1}{8}$ c.c., intermediate dilutions containing $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., and $\frac{1}{16}$ c.c. of blood were, therefore, made and incubated for Bloods 16, 17, 18, 19. The primary dilutions in Bloods 20 to 25 were made by multiples of 3 from the $\frac{1}{20}$ dilution, i.e., $\frac{1}{60}$, $\frac{1}{180}$, $\frac{1}{540}$, $\frac{1}{1620}$, and $\frac{1}{4860}$. From Blood 26 onwards to Blood 51 by multiples of 2; thus one tube containing 19 c.c. of broth and one of blood remained as the unit 1 c.c. of blood, the other tube of similar contents had 10 c.c. abstracted and was hence left containing $\frac{1}{2}$ c.c. blood, the 10 c.c. removed was added to a 10 c.c. broth tube, the resulting 20 c.c. of mixture well amalgamated, and 10 c.c. then abstracted thus leaving it containing $\frac{1}{4}$ c.c. blood; and thus tubes containing $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, and $\frac{1}{256}$ c.c. respectively of blood were prepared, the intention being to increase these dilutions if *M. melitensis* was ever recovered from the highest, though the first twenty-five bloods drawn had not yielded it in so high a dilution as $\frac{1}{100}$.

These blood dilutions were daily thoroughly well shaken to give the *M. melitensis* an opportunity of emerging from the leucocyte in which it was thought to be most probably lodged, and after 5 days incubation, subcultures on to agar slopes from the respective broth tubes were prepared and incubated at 37° C. These inoculations of agar slopes were repeated when considered necessary and no blood dilution was abandoned as unfruitful till it had been incubating at least 11 days.

The Petri dish method was worked side by side with the broth enrichment method for the first seventeen cases, afterwards it was abandoned. The original idea was that the number of colonies of *M. melitensis* appearing could be taken as an index of the quantity of *M. melitensis* in the measured quantity of blood taken. It was found quite easy by inclining the plate to get the blood put on the agar surface to spread itself quite evenly over the whole area of agar forming a very thin layer, but when, as in Cases 10, 12, 14, and 16,

M. melitensis was recovered by the broth method, while the plate method failed to show it, time was felt to be too valuable to persevere with the latter.

Of the details given in the tabulated results some explanation is necessary. In the column headed nation and sex, E = English, M = Maltese, A = Army, N = Navy, F = Female, and as the only female patients from which blood was taken were Maltese, the sex is specified only for that nationality, thus M.M. = Maltese male, and M.F. = Maltese female. The English patients were all male.

The temperatures given preceding drawing of blood are for the few days immediately prior to drawing of blood, the last being the temperature on day of abstraction of blood, these are given as follows : $\frac{10}{9}$ the upper temperature being the morning the lower the evening temperature. In some of the Maltese cases where, owing to the frequent unexpected discharge of patients at their own request prompt action was necessary, blood was taken very soon after admission, and in such cases temperature for only 1 or 2 days could be so given.

The day of disease is enumerated from the first onset of symptoms attributable to the fever.

The time at which blood was drawn is given, it was noted with the intention of seeing if any difference in result would appear between blood taken in the forenoon and that taken in the evening, the patient's temperature at time of drawing is here given also.

The agglutination test was applied by me to all samples of blood drawn, to independently confirm the diagnosis of Malta Fever, and after working out eighteen bloods, it was felt it would be of interest to know the *limit dilution* which would agglutinate a standard fresh agar growth of *M. melitensis* to see if there was any relation between amount of *M. melitensis* obtained from a given blood and the agglutinating power of the latter. The standard taken is an arbitrary one, being that agglutination should be unmistakably marked under the $\frac{2}{3}$ inch objective, 15 minutes after the mixing of *M. melitensis* emulsion and diluted serum, invariably comparison was made with a control.

In the column headed Recovery of *M. melitensis* the sign + means recovery, and the sign - means no recovery.

Smallest quantity of blood means the smallest quantity calculated from the highest broth dilution yielding *M. melitensis* and the amount of blood therein contained.

The following tests were invariably applied to each recovery of *M. melitensis* before it was entered as such in the laboratory records :—

1. Growth on agar slope should be that characteristic of *M. melitensis*.
2. Size and appearance of cocci in film stained with dilute carbol-fuchsin should be characteristic.

| No. of case. | Nation and sex. | Age. | Stage of the fever. | Temperature of patient for few days preceding bleeding. | Day of disease. | Time of bleeding and patient's temperature. | Maximum dilution of patient's blood saving aggl. | Recovery of <i>M. melli-tensis</i> . | Smallest quantity of blood giving <i>M. melli-tensis</i> . |
|--------------|-----------------|------|--|---|-----------------|---|--|--------------------------------------|--|
| 1 | E. A. | 37 | { Had 3 waves. Now convalescent | ° F. Normal for preceding 20 days | 98th | 12.30 noon, N. | Aggl. | + | 1 c.c. |
| 2 | " | 31 | { Had 1 wave | Normal for preceding 7 days | 30th | 12.30 noon, N. | Aggl. | - | |
| 3 | " | 28 | { T. never normal since admitted; a long severe case | E.T.'s = 101, 100, 99, 99 | 101st | 12.30 noon, N. | Aggl. | - | |
| 4 | " | 31 | { Mild case, 4 waves .. | Normal for preceding 30 days | 108th | 12.30 noon, N. | Aggl. | - | |
| 5 | M. M. | 40 | { End of 4th wave .. | 99, 98, 99 93, 98'4, | 74th | Noon, N. | Aggl. | - | |
| 6 | " | 22 | { In 1st wave | 101'6, 101, 101 102'4, 102'6, | 15th | Noon, 100° 8 | Aggl. | - | |
| 7 | " | 24 | { End of 2nd wave .. | 99'4, 99, 98, 98 101, 99, 99, | 49th | 11.30 noon, N. | Aggl. | + | 1½ c.c. |
| 8 | M. F. | 56 | { End of 2nd wave .. | N. N. N. N. 100, 99'6, 99, | 30th | 11.30 A.M., N. | Aggl. | + | 1½ c.c. |
| 9 | " | 28 | { Nearing end of 2nd wave | 99, 99, 99'6, 100'4 102, 101'6, 101'2, | 41st | Noon, 100° | Aggl. | - | |
| 10 | " | 18 | { No information ... | N. 99, 98, 101 101, 101, 101'6, | 37th | 11.45 A.M., 100° 6 | Aggl. | + | 1 c.c. |
| 11 | M. M. | 31 | { Now in hospital for orchitis. Had fever 8 months ago | Normal for months | 240th | Noon, N. | Aggl. ½ | - | |

| | | | | | | | | | |
|----|-------|----|---|--|------|-------------------|---------------------|---|--------------------|
| 12 | " | 38 | In 1st wave | $\frac{100}{100}, \frac{99}{101}, \frac{99}{100}, \frac{100}{102}$ | 10th | 5.15 P.M., 102° | Aggl. | + | $\frac{1}{2}$ c.c. |
| 13 | " | 30 | In 1st wave | $\frac{98}{102}, \frac{99}{99}$ | 9th | 5.30 P.M., 99° | Aggl. | - | $\frac{1}{2}$ c.c. |
| 14 | " | 47 | { Ill at home 3 months. Now admitted because worse | $\frac{100}{102}, \frac{101}{101}, \frac{100}{101}, \frac{100}{99}, \frac{100}{94}$ | 95th | 5.0 P.M., 99°·4 | Aggl. | + | $\frac{1}{2}$ c.c. |
| 15 | " | 25 | { Nearing end of 1st wave | $\frac{101}{101}, \frac{101}{99}, \frac{99}{92}, \frac{99}{92}, \frac{99}{92}, \frac{99}{92}$ | 31st | 5.20 P.M., N. | Aggl. | - | $\frac{1}{2}$ c.c. |
| 16 | " | 22 | Middle of 3rd wave | $\frac{103}{103}, \frac{102}{103}, \frac{102}{103}, \frac{102}{103}, \frac{102}{103}, \frac{102}{103}$ | 38th | 5.10 P.M., 103°·2 | 1000 | + | $\frac{1}{2}$ c.c. |
| 17 | " | 36 | In 1st wave | $\frac{100}{100}, \frac{102}{101}, \frac{103}{103}, \frac{101}{101}, \frac{101}{101}, \frac{101}{101}$ | 17th | 5.25 P.M., 101°·6 | Aggl. $\frac{1}{2}$ | - | $\frac{1}{2}$ c.c. |
| 18 | " | 29 | In 1st wave | $\frac{102}{102}, \frac{103}{103}, \frac{103}{103}, \frac{103}{103}, \frac{103}{103}, \frac{103}{103}$ | 9th | 5.30 P.M., 103°·4 | $\frac{1}{2}$ | + | $\frac{1}{2}$ c.c. |
| 19 | M. F. | 44 | In 1st wave | $\frac{102}{103}, \frac{104}{103}, \frac{103}{103}, \frac{101}{103}, \frac{101}{103}, \frac{101}{103}$ | 15th | 5.45 P.M., 100°·5 | $\frac{1}{2}$ | + | $\frac{1}{2}$ c.c. |
| 20 | E. N. | 22 | In 2nd wave | $\frac{100}{103}, \frac{100}{103}, \frac{100}{103}, \frac{100}{103}, \frac{100}{103}, \frac{100}{103}$ | 22nd | 10.30 A.M., 100° | 300 | - | $\frac{1}{2}$ c.c. |
| 21 | " | 32 | In 1st wave | $\frac{101}{102}, \frac{102}{103}, \frac{102}{97}, \frac{102}{96}, \frac{102}{96}, \frac{102}{96}$ | 28th | 10.20 A.M., N. | 1000 | - | $\frac{1}{2}$ c.c. |
| 22 | M. M. | 15 | In 1st wave | $\frac{103}{104}, \frac{103}{102}, \frac{102}{105}, \frac{102}{105}, \frac{102}{105}, \frac{102}{105}$ | 11th | 5.30 P.M., 103°·8 | $\frac{1}{2}$ | + | $\frac{1}{2}$ c.c. |
| 23 | " | 24 | { In 1st wave. Con- tinuous fever | $\frac{102}{102}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}$ | 31st | 5.40 P.M., 102° | $\frac{1}{2}$ | + | $\frac{1}{2}$ c.c. |
| 24 | " | 29 | In 1st wave | $\frac{103}{103}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}, \frac{102}{102}$ | 13th | 5.50 P.M., 99°·4 | 1000 | - | $\frac{1}{2}$ c.c. |
| 25 | M. F. | 39 | In 1st wave | $\frac{99}{101}, \frac{99}{101}, \frac{99}{101}, \frac{99}{101}, \frac{99}{101}, \frac{99}{101}$ | 18th | 6.10 P.M., 101°·4 | 1000 | - | $\frac{1}{2}$ c.c. |
| 26 | M. M. | 37 | In 1st wave | $\frac{100}{102}, \frac{100}{102}, \frac{100}{102}, \frac{100}{102}, \frac{100}{102}, \frac{100}{102}$ | 12th | 5.0 P.M., 102° | 1000 | + | $\frac{1}{2}$ c.c. |
| 27 | " | 23 | In 1st wave | $\frac{100}{100}, \frac{101}{101}, \frac{101}{101}, \frac{101}{101}, \frac{101}{101}, \frac{101}{101}$ | 7th | 5.15. P.M., 102° | $\frac{1}{2}$ | + | $\frac{1}{2}$ c.c. |

| No. of case. | Nation and sex. | Age. | Stage of the fever. | Temperature of patient for few days preceding bleeding. | Day of disease. | Time of bleeding and patient's temperature. | Maximum dilution of patient's blood swing aggl. | Recovery of <i>M. melitensis</i> . | Smallest quantity of blood giving <i>M. melitensis</i> . |
|--------------|-----------------|------|---------------------------------|--|-----------------|---|---|------------------------------------|--|
| 28 | M. F. | 24 | { In 1st wave. Continuous fever | 99.6, 101.2, 101, 99, 100, 102.8, 103, 101.2, 101.8, 103.2 | 32nd | 5.30 P.M., 102° | 1300 | — | |
| 29 | " | 44 | { In 2nd wave | 101, 99, 100.2, 100.2, 101.4, 102.4, 102.6, 102, 101.6, 102 | 56th | 5.45 P.M., 101° 8 | 1000 | + | 1 c.c. |
| 30 | E. A. | 23 | { In 1st wave | 101, 100.6, 100.6, 100.5, 100.4, 102.3, 102.8, 102.8, 101.8, | 15th | 11.0 A.M., 100° | 400 | + | 1/5 c.c. |
| 31 | " | 27 | { In 1st wave | 100, 101.6, 101.6, 101.6, 102, 101, 103, 103, 104.2, ... | 22nd | 11.15 A.M., 101° 8 | 400 | — | |
| 32 | " | 37 | { In 1st wave | 99, 99.6, 101, 102, 99.4, 101.6, 103.4, 102.6, 103, | 36th | 11.30 A.M., 99.8 | 3000 | + | 1 c.c. |
| 33 | M. M. | 55 | { In 1st wave | 100, 99.4, 99.2, 99.4, 101, 100, 100.6, 100.6, 102.2, 101, ... | 10th | 5.10 P.M., 101° | 400 | + | 1 c.c. |
| 34 | " | 17 | { In 1st wave | 101, 101, 99.2, N, 99.2, 101, 101, 101, 102, 100.6, ... | 8th | 5.30 P.M., 103° 8 | 100 | + | 1/5 c.c. |
| 35 | " | 38 | { In 1st wave | 101, 101, 101, 102, 100.6, ... | 18th | 5.0 P.M., 100° 6 | 400 | + | 1 c.c. |
| 36 | " | 31 | { In 1st wave | 101, 101.8, 103.8, 102.2, 102.4, 101.8 | 7th | 5.10 P.M., 102° 2 | 1/4 | + | 1/5 c.c. |
| 37 | " | 43 | { No information | 102.6, 102.8, 101, 102, 102.6, 103, 104, 103.8, 103.4, 102 | 36th | 5.20 P.M., 102° 4 | 1000 | + | 1/5 c.c. |
| 38 | E. A. | 22 | { Middle of 2nd wave | | 26th | 5.40 P.M., 102° | 1000 | + | 1/5 c.c. |

| | | | | | | | | | |
|----|-------|----|------------------------------------|-------------------------------------|--------|-------------------|------|---|---------------------|
| 39 | " | 21 | { Near end of 1st wave | { 104, 103, 101, 98.4, 98.4 | { 9th | 11.0 A.M., 98.4 | 1.00 | + | $\frac{1}{8}$ c.c. |
| 40 | " | 22 | { Near end of 3rd wave | { 98.6, 99.2, 99.2, 99.6, 99.2 | { 57th | 11.10 A.M., 99.2 | 1.00 | - | |
| 41 | " | 38 | { Near end of 2nd wave | { 98.8, 98.6, 98.4, 99, 99 | { 48th | 11.20 A.M., 99.4 | 1.00 | - | |
| 42 | " | 32 | { Near end of 2nd wave | { 100.8, 100, 100.2, 100.2, 100.2 | { 55th | 11.30 A.M., 99.6 | 1.00 | + | $\frac{1}{8}$ c.c. |
| 43 | E. N. | 26 | In 1st wave | { 102.3, 102.7, 101.5, 102, 102 | { 15th | 10.30 A.M., 99.6 | 1.00 | + | $\frac{1}{16}$ c.c. |
| 44 | " | 27 | In 1st wave | { 99.4, 99.4, 99.6, 98.6, 98.6 | { 17th | 10.20 A.M., N. | 1.00 | - | |
| 45 | E. A. | 31 | { Nearing end of 2nd wave | { 98.6, 98.4, 98.5, 98.4, 98.8 | { 41st | 9.50 A.M., 99.0 | 1.00 | + | $\frac{1}{16}$ c.c. |
| 46 | " | 27 | { Nearing end of 3rd wave | { 100, 100.6, 100.2, 101, 101 | { 69th | 10.5 A.M., 99.0 | 1.00 | - | |
| 47 | " | 20 | Middle of 1st wave | { 102.2, 102, 101.2, 101, 101 | { 25th | 11.10 A.M., 101.0 | 1.00 | + | $\frac{1}{16}$ c.c. |
| 48 | " | 20 | { Now commencing 2nd wave | { 103.4, 103.4, 103.2, 103, 101 | { 28th | 11.20 A.M., 101.0 | 1.00 | - | |
| 49 | " | 38 | Height of 1st wave | { 101.6, 103.5, 103.4, 103.2, 101 | { 22nd | 11.30 A.M., 102.0 | 1.00 | + | 1 c.c. |
| 50 | " | 20 | Middle of 1st wave | { 103.6, 104.6, 104.7, 105.6, 105.6 | { 42nd | 10.50 A.M., 100.4 | 1.00 | + | $\frac{1}{16}$ c.c. |
| 51 | " | 40 | { Only now nearing end of 1st wave | { 102.2, 103.2, 102.7, 102.8, 99.2 | { 55th | 11.5 A.M., 99.4 | 1.00 | + | $\frac{1}{8}$ c.c. |

3. Non-staining with Gram.
4. No development of gas, acidity or coagulation when grown in litmus milk, but production of alkalinity.
5. No production of acidity, but production of alkalinity when grown on glucose-litmus-agar.
6. Mobility in hanging drop merely Brownian, no translation from portion to portion of field.
7. Should be agglutinated, visibly to the naked eye by a $\frac{1}{500}$ dilution of a pure animal serum, obtained by inoculating an animal (rabbit and monkey were both used), with a pure standard growth of *M. melitensis*. Comparison with a control was always made, and the two submitted to my fellow-worker, Major Horrocks, R.A.M.C., at the next bench, and unless he concurred as to the indubitable nature of the reaction it was not accepted.

There has been considerable difficulty in extending this series of blood examinations even so far as it has gone. Patients did not like it; some consented freely, others reluctantly, and their physicians were not prepossessed in favour of it either. One would have liked to have taken a few cases and taken specimens of blood every day or every other day, and so ascertained when the *M. melitensis* appeared in and disappeared from the peripheral blood during the whole course of the fever; but it was found impossible to accomplish this. Only with one patient did I succeed in getting blood twice for examination; the first time reported as No. 6, result negative; and the second time as No. 16, result positive.

As regards syringes, I found it simplest to sterilise them in the autoclave at 120° C. The needles I found did best sterilised in pure olive oil at about 140° C.; this prevented rust and their points retained their primitive sharpness. I also found blood was obtained with greater facility if the needle were passed into the vein from the bend of the elbow towards the hand, so that blood entered the syringe in the direction of natural flow.

This method of taking blood from the median basilic vein and incubating it in broth was apparently first described by Dr. Jules Courmont, at a meeting of the Société Médicale des Hôpitaux de Paris, December 27, 1901, who applied it successfully in nine cases of Typhoid Fever in which he recovered *B. Typhosus* from the peripheral blood. I saw the method in application in Vidal's Clinique in the Hôpital Cochin in Paris in the winter of 1902-3, there studied it and applied it successfully to the recovery of *M. melitensis* from the peripheral blood of Malta Fever patients in the summer of 1903. So far as I know the dilution method to determine the smallest quantity of fluid containing the micro-organism has not hitherto been applied in the recovery of micro-organisms from the circulating blood, though it is classical in the history of the bacterial analysis of water. It has

obvious advantages over the plating method, a most important one being that as in Blood No. 27, there were only nine growths representing the nine dilutions $1, \frac{1}{2}, \frac{1}{4}, \frac{1}{8}, \frac{1}{16}, \frac{1}{32}, \frac{1}{64}, \frac{1}{128}, \frac{1}{256}$ c.c., to examine and put through the various tests for *M. melitensis*; whereas had 1 c.c. of this blood been plated out, it would presumably have yielded over 200 colonies, which would have required verification individually, as unfortunately all the colonies found in a blood plate are not necessarily of the same kind, and one cannot apply the principle *Ex uno omnes discere*.

Conclusions.

1. *M. melitensis* exists in the blood of patients in relatively very small amount, the smallest quantity of blood in which it has been found, $\frac{1}{256}$ c.c. is practically the equivalent of 4 c.mm. and as 1 cubic millimetre of blood = 5,000,000 corpuscles, and if *M. melitensis* is never found in association with less number of corpuscles than 20,000,000 it is obvious there is no comparison between this and such a disease as anthrax, for instance, in which in the blood the number of bacilli has been found in some cases equalling the number of corpuscles. This has an important bearing on the question of transmission of infection by mosquitoes.

2. No definite relation can be established between any given stage of the disease and the presence of *M. melitensis* in the blood. It has been found as early as the 7th day Cases 27 and 36, and as late as the 95th and 98th day Cases 1 and 14. It has been found in the majority of cases when the temperature of the patient has been raised, but it has been also present in convalescence (Case 1), and when temperature has been normal (Cases 7, 8 and 39), for several days, but it has also not been found when the temperature was high, Cases 6, 25, 28, 31, and 48.

3. There is some indication of a diurnal variation in its presence in the blood, out of 29 cases where blood was taken in the forenoon between the hours of 10 and 12.30, it was present in 14, absent in 15. Out of 22 cases where blood was drawn in the evening between 5 and 6.30 p.m. it was present in 16, absent in 6; a ratio of almost 3:1 in favour of the evening.

4. No relation can be established between the agglutinating power of a patient's blood for *M. melitensis* and the amount of the latter present in the blood, most of the cases in which it was found had a high agglutinating power, but one of the cases in which *M. melitensis* was found in one of the smallest quantities of blood, $\frac{1}{128}$ c.c. (Case 37) only agglutinated in a $\frac{1}{10}$ dilution, as against another in which it was found in $\frac{1}{256}$ c.c., in which there was agglutination with a dilution of 1 in 1000, and others where it was not found at all where there was agglutination in a dilution of $\frac{1}{1800}$, Cases 41, 44 and 48.

5. In some of the cases the *M. melitensis* was found to have skipped some of the dilutions, for instance, in Case 34, where the dilutions proceeded by powers of 2 from 1 to 256, *M. melitensis* was found in the 1 c.c., $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., dilutions, absent from the $\frac{1}{8}$ c.c. and $\frac{1}{16}$ c.c. dilutions, present in the $\frac{1}{32}$ and $\frac{1}{64}$ dilutions, absent in the rest. In Blood 37, in which same series of dilutions were made, *M. melitensis* was present in all up to the $\frac{1}{64}$ c.c. inclusive, with the exception of the $\frac{1}{16}$, these were the only two cases out of the fifty-one in which this jumping took place. It is certainly not due to inadequate mixing of the dilutions, for the primary blood dilution, from the moment the blood got into it, which was instantly on the needle being withdrawn from the vein, was agitated vigorously until a considerable froth was on its surface, and so on with the succeeding dilutions. It may possibly be due to the small quantity of *M. melitensis* in the blood, or to the *M. melitensis* being in some dilutions so phagocytosed as to be unable to escape and multiply.

Examination of Bloods.

Table showing in chronological order the date of the disease in each case in which blood was taken for bacteriological examination, and the result. The fractions of a cubic centimetre indicate the smallest amount of blood from which *M. melitensis* was obtained; the sign - means no *M. melitensis* was recovered; the days of disease which are not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by 1, 2, 3, or 4 examinations of blood. This has been unavoidable; the number of cases willing to submit to venous puncture was too small to admit of selection; and waiting a few days usually meant losing the case.

| Day of disease. | Recovery and quantity or no recovery. | Day of disease. | Recovery and quantity or no recovery. | Day of disease. | Recovery and quantity or no recovery. |
|-----------------|---|-----------------|---------------------------------------|-----------------|---------------------------------------|
| 1 | | 38 | | 75 | |
| 2 | | 39 | | 76 | |
| 3 | | 40 | | 77 | |
| 4 | | 41 | —, $\frac{1}{16}$ c.c. | 78 | |
| 5 | | 42 | $\frac{1}{128}$ c.c. | 79 | |
| 6 | | 43 | | 80 | |
| 7 | $\frac{1}{8}$, $\frac{1}{32}$ c.c. | 44 | | 81 | |
| 8 | $\frac{1}{64}$ c.c. | 45 | | 82 | |
| 9 | —, $\frac{1}{8}$, $\frac{1}{8}$ c.c. | 46 | | 83 | |
| 10 | $\frac{1}{8}$, 1 c.c. | 47 | | 84 | |
| 11 | $\frac{3}{4}$ c.c. | 48 | — | 85 | |
| 12 | 1 c.c. | 49 | $\frac{1}{10}$ c.c. | 86 | |
| 13 | — | 50 | | 87 | |
| 14 | | 51 | | 88 | |
| 15 | —, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{16}$ c.c. | 52 | | 89 | |
| 16 | | 53 | | 90 | |
| 17 | —, — | 54 | | 91 | |
| 18 | —, $\frac{1}{2}$ c.c. | 55 | $\frac{1}{64}$, $\frac{1}{64}$ c.c. | 92 | |
| 19 | | 56 | $\frac{1}{4}$ c.c. | 93 | |
| 20 | | 57 | — | 94 | |
| 21 | | 58 | | 95 | $\frac{1}{8}$ c.c. |
| 22 | —, —, 1 c.c. | 59 | | 96 | |
| 23 | | 60 | | 97 | |
| 24 | | 61 | | 98 | $\frac{1}{2}$ c.c. |
| 25 | $\frac{1}{32}$ c.c. | 62 | | 99 | |
| 26 | $\frac{1}{16}$ c.c. | 63 | | 100 | |
| 27 | | 64 | | 101 | |
| 28 | —, — | 65 | | 102 | — |
| 29 | | 66 | | 103 | |
| 30 | —, $\frac{1}{10}$ c.c. | 67 | | 104 | |
| 31 | —, $\frac{1}{8}$ c.c. | 68 | | 105 | |
| 32 | — | 69 | — | 106 | |
| 33 | | 70 | | 107 | |
| 34 | $\frac{1}{4}$ c.c. | 71 | | 108 | — |
| 35 | | 72 | | 240 | — |
| 36 | 1, $\frac{1}{64}$ c.c. | 73 | | | |
| 37 | $\frac{7}{8}$ c.c. | 74 | — | | |

Examination of Epidermis of Malta Fever Patients for M. melitensis.

Method.—Patients were selected with temperatures of 100° F. and upwards in different stages of the fever from the 15th to 60th day, epidermis from the arms and flanks scraped away with a sharp sterilised scalpel till the dermis threatened pin-point hæmorrhages, the scrapings put in sterilised capsules, taken to the laboratory and there ground up in a small quantity of sterile normal salt solution — (1 c.c.). From this three successive agar Petris were inoculated with one loopful, to the remainder, 5 c.c. of salt solution was added, and the surface of three other agar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted skin emulsion over each, and the whole incubated at 37° C. for 5 days.

Up to the present this method has been applied to twelve cases.

Discrete colonies of the different micro-organisms usually met with in the skin were obtained in every case, but in none of these plates were colonies of *M. melitensis* ever obtained.

Examination of Sweat from Malta Fever Patients for M. melitensis.

1st Method.—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry 160° C. air) lint placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth tube numbered, dated, and incubated at 37° C. After 5 days' incubation, agar slopes, inoculated zig-zag from each, incubated at 37° C. and examined daily for growth; if sterile, original broth tubes were inoculated with *M. melitensis* returned to incubator for 4 days and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface. Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

2nd Method.—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The collection was done by the sisters in the ward who were supplied with the pipettes ready for use, and instructed how to break off the points and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

3rd Method.—(A modification of the 1st.)—Circles of lint were obtained saturated with critical sweat from Malta Fever patients as in 1st Method, but instead of being incubated in broth tubes were placed each in a 5 c.c. sterile normal salt solution tubes, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading $\frac{1}{2}$ c.c. of it over whole surface, and by describing a centripetal spiral with a loop full of the fluid. Discrete colonies were always thus obtained after incubation at 37° C.

The critical sweats of seven patients have been thus examined without *M. melitensis* having been obtained.

To see if M. melitensis would Pass any Filter.

It was felt it would be of the greatest assistance in isolating *M. melitensis* if advantage could be taken of its small size to separate it from other larger organisms by means of filtration, and I, therefore, experimented with the following filters as described :—

New filters were used for the first time in each case. Obviously the first indication was to find a filter that would pass *M. melitensis* and later to see if *M. melitensis* would come through it from a mixture of microbes. Bougies were all first tested for imperfections by placing in water and applying air under pressure. Chamberland F. was first tried after being sterilised in the autoclave at 155° C. 1 hour. All junctions were luted with paraffin.

July 8. Placed broth emulsion of verified living *M. melitensis* from one agar slope in container, filled up with peptone broth, tightened pinch cock, placed apparatus in incubator at 37° C.

July 9. Broth in flask remains clear, loosened pinch cock and ran in 6 drops from bougie. This was repeated daily till July 30, apparatus being kept in incubator at 37° C. all the time.

July 30. Three agar slopes inoculated with some of filtrate, drawn off with a sterile pipette from flask through side tube and incubated.

August 4. No growth in any of agar slopes. Experiment concluded.

Result.—No *M. melitensis* has either been washed through or has grown through Chamberland F.

2nd Filtration Experiment with M. melitensis.

July 7. Took Chamberland F. bougie, tested for imperfections in water with air under pressure, cut off porcelain end, heated resulting cylinder to redness in moufle; fitted up to act as filter, first sterilising all glass parts at 180° C. for 30 minutes, then sterilised apparatus in autoclave 30 minutes at 120° C., and finally luted junctions with paraffin.

July 8. Placed emulsion of living tested *M. melitensis* (emulsion in broth from growth on one agar slope) in cavity of bougie and filled up with peptone broth, removing glass rod in rubber cork to allow of escape of contained air; replaced plug of wool in end of tube; replaced glass rod; and placed apparatus in incubator at 37° C.

July 9. Broth coming through filter into cavity of test-tube, displaced air escaping by tube B which had been also plugged with cotton wool.

July 27. Apparatus has now been in incubator 18 days. Inoculated three agar slopes with filtrate obtained by means of a sterile pipette passed down tube B, and placed these in incubator at 37° C.

July 31. Agar slopes have now been in incubator 4 days and remain without growth.

Result.—*M. melitensis* does not pass Chamberland F.

3rd Filtration Experiment with M. melitensis.

To see whether *M. melitensis* will pass any of three Berkefeld filters N., V., and W., of differing porosities (these were obtained from the Lister Institute).

One of each porosity was taken, tested in water with compressed air, sterilised, and fitted up, glass container being first sterilised by boiling in water and then in hot air 1 hour at 160° C. An air pass being arranged in rubber collar to allow of air displaced by filtrate escaping from container. Then the whole sterilised in autoclave at 115° C. for $\frac{1}{2}$ hour.

August 7. Eight cubic centimetres of 5 days' old verified broth growth of *M. melitensis* placed in each bougie with a sterile pipette.

August 8. Some filtrate in container, 8 c.c. more of same broth culture placed in each bougie.

August 9. Five cubic centimetres more of same culture in each bougie.

August 10. Five cubic centimetres more of same culture in each bougie.

August 11. Now placed in incubator at 37° C.

August 22. Inoculated two glucose-litmus-agar slopes from contents of each container. Placed in incubator at 37° C.

September 3. No growth in any of slopes of 22nd. Experiment concluded.

Result.—*M. melitensis* will not pass any of Berkefeld filters N., V., or W.

4th Filtration Experiment with M. melitensis.

To see if *M. melitensis* will grow through Berkefeld filters N., V., or W.

One of each porosity taken and treated as in 3rd filtration experiment, and sterilised in autoclave.

August 14. Placed in each bougie with a sterile pipette 5 c.c. of a verified 4 days' broth culture of *M. melitensis*.

August 15. Five more cubic centimetres of same *M. melitensis* broth culture placed in each bougie.

August 16. Filters now working well; V. cylinder being one-third full of filtrate with its bougie immersed in same for $\frac{1}{2}$ inch, W. and N. bougies are only just touching surface of filtrate, so 5 c.c. more of *M. melitensis* broth culture placed in each bougie W. and N.

August 17. N. receiver now half full of filtrate, bougie being

immersed for $\frac{3}{4}$ inch. More *M. melitensis* broth culture added to W. bougie only.

August 18. W. bougie now well immersed in filtrate. Placed all three in incubator at 37° C.

August 23. Filtrate in N. and W. decreasing in bulk by evaporation through wool plug. Placed more *M. melitensis* broth culture inside these two bougies. Returned to incubator at 37° C.

August 29. Broth filtrates from B., V., and W. have now been incubating at 37° C. for 11 days, bougies being immersed, and remain free from turbidity. Inoculated two agar slopes from each and placed in incubator at 37° C.

September 3. No growth in any of slopes of 29th. Experiment concluded.

Result.—*M. melitensis* will not grow through any of Berkefeld filters N., V., or W.

To Produce a Pure Agglutinating Serum for Testing M. melitensis (or Growths Suspected to be M. melitensis) by Inoculating Rabbits with M. melitensis.

At first, serum brought by Major Horrocks from Gibraltar, and obtained from a rabbit so inoculated, by him was used for testing all new growths thought to be *M. melitensis*. Later serum obtained from an inoculated monkey, and from the second rabbit in the following three experiments was used :—

1st Rabbit.

June 18. A healthy-looking rabbit was taken, of weight 1310 grammes, and its blood examined for agglutinating action on *M. melitensis*. None was found, and it was injected subcutaneously with $\frac{1}{2}$ c.c. of a 24 hours' growth of *M. melitensis* in broth at 37° C. (verified).

June 25. Agglutination $\frac{1}{10}$ under $\frac{2}{3}$ in obj.

June 28. " $\frac{1}{10}$ " and it was injected under skin of back with a 4 days' growth of *M. melitensis* on one agar slope (verified) emulsified in broth.

July 3. Rabbit found dead. *Post-mortem*. There was slight congestion of intestines, spleen, and peritoneal vessels; liver somewhat patchy, heart normal. Stomach full of green food. No *post-mortem* cultures were attempted as animal had apparently been dead 12 to 16 hours.

2nd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope at 37° C., made into an emulsion with $2\frac{1}{2}$ c.c. broth, 1 c.c. of this

injected under skin of back of a fawn and white rabbit weighing 1460 grammes.

July 13. Serum agglutinates in a dilution of $\frac{1}{10}$ *M. melitensis* faintly (microscope $\frac{1}{8}$ obj.); all growth on one agar slope (3 days) of *M. melitensis* (from spleen of man) emulsified in broth and injected subcutaneously.

July 21. Serum in a dilution of $\frac{1}{320}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 24. Serum in a dilution of $\frac{1}{60}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 27. Serum in a dilution of $\frac{1}{800}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

Injected growth from two-agar slope of *M. melitensis* (spleen of man), July 27.

July 31. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

August 4. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* visibly to naked eye. Blood had been drawn as required from July 22 onwards.

August 8. Agglutinates *M. melitensis* $\frac{1}{1000}$ visible to naked eye; rabbit now bled to death under ether from carotid by cannula into sterile test-tubes. After separation of serum latter diluted to $\frac{1}{20}$ with sterile salt solution containing $\frac{1}{2}$ per cent. carbolic acid put up in sterile sealed glass capsules and preserved.

Post-mortem.—All organs appear healthy, spleen enlarged. Inoculated to agar slopes each from spleen, liver, kidney, heart's blood and urine.

August 11. Growth on tubes inoculated from *spleen* and *kidneys*, verified as *M. melitensis*. No growth on slopes from liver, heart's blood, and urine.

August 13. Still no growth on slopes from liver, heart's blood, and urine. Experiment concluded.

3rd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope made into emulsion with $2\frac{1}{2}$ c.c. broth, and 1 c.c. of this injected under skin of black and white rabbit, 11 A.M., July 4.

July 9. Serum does not agglutinate *M. melitensis*.

July 13. Serum in a dilution of $\frac{1}{10}$ agglutinates *M. melitensis* ($\frac{1}{4}$ obj. microscope). One agar tube *M. melitensis* from spleen of man emulsified and injected.

July 15. Rabbit died at 4 P.M. A *post-mortem* was made and liver found enlarged and studded with cheesy tubercles the size of peas. Other organs apparently healthy. Two agar slopes inoculated from each. Heart's blood, liver, kidney, and spleen; 2 c.c. of urine taken

with sterile pipette and put in 19 c.c. broth. All
in 37° C.

No growth on any of slopes; incubated agar slopes from
urine.

July 19. Growth on slope from urine broth; found to be a short
thick bacillus.

July 21. Heart's blood, kidney, liver, and spleen slopes have now
been incubated 6 days. No growth on any of them. Experiment
concluded.

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REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART II.

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REPORT UPON THE GENERAL SANITARY CIRCUMSTANCES OF THE MALTESE ISLANDS, WITH SPECIAL REFERENCE TO THE PREVALENCE OF MEDITERRANEAN FEVER THEREIN.

PART I.—GENERAL SANITARY SURVEY.

PART II.—MEDITERRANEAN FEVER.

PART III.—OUTBREAK OF MEDITERRANEAN FEVER IN THE ESSEX REGIMENT.

PART IV.—GENERAL SUMMARY AND CONCLUSION.

By DR. RALPH W. JOHNSTONE, Medical Inspector Local Government Board.

Brief Description of the Maltese Islands.

These islands include Malta, Gozo, Comino, and Cominetto. The two latter are islets, and, save for the hospital for exotic disease on Comino, with its caretaker, are uninhabited.

The island of Malta is about 17 miles long by 8 wide, having an area of 91 square miles. At the census of 1901 the actually resident population was returned as 176,127. This includes a garrison of about 11,000 men. There were besides about the same number of men on the fleet who were not included.

Gozo is about 9 miles by $4\frac{1}{2}$, with an area of 26 square miles. Its population was 20,002 in 1901.

Both islands are covered by low hills, which, with their intervening valleys, leave little or no flat surface. In Gozo the hills are more abrupt, but in neither island is the highest point more than 760 feet above sea level. Every available inch of land is cultivated, but the fields are shut in by high stone walls, which gives the country its characteristic stony and sterile appearance. There are no rivers.

Geologically the formation is an Upper Coralline limestone, under which are successive beds of greensand and marl, overlying a Globigerina limestone (often locally called calcareous sandstone), which again overlies a Lower Coralline limestone. Practically, all the inhabited part of Malta is denuded down to the Globigerina limestone, with occasional outcroppings of the Lower Coralline formation, and one or two patches of alluvium. The north-western and more elevated part of Malta is, however, covered by the Upper Coralline limestone, as is also the higher ground in Gozo.

PART I.—GENERAL SANITARY SURVEY OF THE MALTESE ISLANDS.

Density of Habitation.—The population enumerated on land at the census of 1901 showed an average density of 1671 persons to the square mile, or in Malta 1926 to the square mile, and in Gozo 775. The population was further classified into (a) persons living within the fortified towns—urban area, 55,298 persons to the square mile; (b) persons living close to the fortified towns—suburban area, 3976 persons to the square mile; and (c) persons living in the districts away from the fortified towns—rural area, 749 persons to the square mile. In Senglea, a part of the urban area, the density was as high as 183,932 persons per square mile, and in Sliema, part of the suburban area, 153,297. In addition, the greater part of the rural population in Malta is housed in villages where the density of population is often higher than in many English cities—Mellieha, for instance, has a density of 147,312 per square mile. Overcrowding of houses upon area is supplemented by overcrowding of persons in houses. Taking as a standard of overcrowding more than two persons living in one room in a tenement consisting of less than five rooms, there are 27 per cent. of the total number of persons in the Maltese islands who are living in overcrowded dwellings. (The percentage in England and Wales at the census of 1901 was 12·2.) This overcrowding is largely contributed to by the number of Kerreyas, or common lodging-houses which exist, especially in the fortified towns. In Valetta, with a total population of about 24,000 persons, more than 5000 persons live in Kerreyas. The population is rapidly increasing. In the decade 1892 to 1901 the Maltese-born population increased 11·6 per cent., other British subjects 12·5 per cent., and foreigners 37·1 per cent.; while each year the population around Valetta, and its harbours, especially tends to increase in density.

Dwellings.—All the dwellings are constructed of stone, generally in two storeys with a flat roof, which is utilised to collect rain-water. In the country districts window space is usually inadequate, sometimes, indeed, altogether absent. Owing to the porous nature of the local stone, the older dwellings, where the walls are not properly protected by copings and damp-proof courses, are said to be damp in winter. Most houses have a small yard or garden, in which is found the mouth of the underground tank used for storing rain-water from the roof. The pavement of the yards is nearly always porous, and is often cracked or defective near the tank mouth, where it is most used. The yard surfaces are very often strewn with refuse and the droppings of birds and animals, or soiled with slop water; they are usually drained to the street gutter. The flooring of rooms is generally constructed of porous stone, which forms a fine dust with surface wear. Rabbits, hens, cats, and dogs are kept in the houses, and sheep and cattle are housed in out-buildings, which usually abut on the dwelling-house.

Roads.—The roads are repaired by the Government, except a few which are repaired by the military authorities. Main roads are kept in good order, but owing to the friability of the local stone used as metalling, there is much dust. Bye-roads are often almost impracticable for wheeled vehicles. In the fortified towns scavenging is done by men in the employment of the Government, and surface water drains are provided. Outside the fortified towns street scavenging is neglected.

Excrement Disposal.—The method most generally employed is what is known as the hand-flushed water-closet. This closet is usually a long hopper basin with a syphon trap below, in connection with a cesspit or a sewer. The closet is, as a rule, used for emptying excrement and slops into. It is seldom used in any other way. It is placed in the most unexpected positions, sometimes in the open yard in a niche in the wall, frequently in the kitchen a foot or two from the cooking apparatus, or it may be in a small cupboard (1' × 2' usually) in the external wall of the house, or in the steps leading to the entrance hall from the front door, and sometimes even in the open street. It is very exceptionally found in a special room or in a position where it is likely to be used in the usual manner. In those poorer class houses which have got a special room for the water closet, the room serves often also as a larder or food store. The hand-flushing of these closets is conspicuous mainly by its absence. Water has always been a precious commodity in Malta, and is used sparingly; in addition cesspits are emptied at the owner's expense, at the high rate of 1s. 6d. per 100 gallons. The consequence is that one rarely sees a water-closet basin even moderately clean. They are usually caked with filth, and the surroundings fouled with fecal matter. Again, when a poor proprietor owns a field or garden, he often ceases to make use of the closet, in order to avoid the cost of emptying, or because he values the excrement as manure. As result, the water in the trap evaporates, and cesspit or sewer air gains access to his premises.

The next most frequent method is what may be called the misbla system. A misbla is a dung heap, and it may be placed in the garden or yard, or more frequently in an outhouse adjoining the living rooms, or in an ordinary room in the house. Here all the excrement of the family is carefully preserved for removal to the fields. The abominable stench that may be caused in a dwelling by this system is not easily described. Occasionally a cellar is used as a misbla, and privy seats are placed in the room above. The vessels which are used to convey excrement from the bedrooms to its destination are often left in the house unemptied for several days. On one occasion on asking to see the vessel used for conveying excrement to the garden, I was shown the bucket in which the vegetables for the family dinner were being washed.

There are a few privy middens, and a few modern properly flushed water-closets. The garrison are provided with trough latrines, flushed once or twice a day, but owing to the feebleness of the flush and the defective pattern of latrine used, were often found overfull and offensive. In places where the latrines were flushed by a rush of water through the trough, a filthy and offensive residue was left behind after the flushing, and in places where the basins were flushed from above into the trough, the feeble trickle of water provided was entirely inadequate. The wooden seats of the military latrines are constructed a few inches above the iron surrounding the opening to the basin, with the result that urine is liable to soil the flat iron surface and dry there, after which the dried residue may be blown about by currents of air.

The naval latrines, or "heads," on board ship are of a better pattern, better flushed, and lack the iron cover underneath the wooden seat. The officers' water-closets, which are used also as urinals, are, from their height above the floor, peculiarly liable to contamination of the floors with urine.

Little effort is made to prevent the pollution of open spaces and walls by excrement and urine. The spaces around the landward fortifications of Floriana are especially liable to fouling, on account of the habits of the country people who pass every day into Valetta. Public urinals sometimes have no water supply, and thus become very offensive.

House Refuse Disposal.—There is no regular system for collection of house refuse in Malta. Certain men with carts call at the houses in the morning and take away house refuse for use as manure, but they are in no way bound to take it, and instances arise where they decline to remove refuse containing material not likely to be useful, such as tin cans and broken bottles. Ash-pits or bins are almost unknown. Refuse is thrown into the garden or into the street. Dead animals, vegetable refuse, and other filth may often be seen in the streets, and many complaints have been made by the better class inhabitants on this score. The police, however, seem powerless to enforce their regulations in the matter.

Owing to the lack of proper flushing the house drains and soil pipes become caked with filth, and complaints of smell arising from the inlet ventilators of house drains are frequent.

Sewers.—In some of the towns, for in Malta the villages are really small towns, quite compact and lacking in open spaces, there are sewers which have existed for centuries. They are built of, or cut in the porous rock, being more in the nature of galleries than sewers, and they are not in any way rendered impervious. They act as elongated cesspools where the liquid sewage gradually soaks away into the rock, leaving a semi-solid residue. Such, for instance, is the system at Birchirchara, Victoria, and formerly at Curmi.

Modern sewers exist at Valetta, Cospicua, Senglea, Vittoriosa, Calcare, Misida, Curmi, and Sliema, and are in course of construction at Notabile and Rabato, and at Hamrun. Many of the houses abutting on the harbour still drain into it, so that when the fleet is in, and many ships are discharging their sewage into the Grand Harbour, the water is liable to become visibly polluted with excremental matter. Since there is very little tide, this may become a serious nuisance.

Cesspits, in the case of new houses, are built under the street and are ventilated and cemented. In the old houses the cesspits are often under the dwelling rooms and unventilated. Very frequently they are placed close to the water tanks. Many old cesspits are never emptied, since they are not impervious, and it is to be feared that new cesspits are occasionally tampered with so as to render them pervious, after they have been passed by the sanitary authority. Cesspits are emptied by means of a pumping engine into iron tank carts. The contractor who undertakes this work is empowered to charge at the rate of 1s. 6d. per 100 gallons.

Water Supply.—There is a public water supply laid on to every village in Malta except Mellieha. The water is derived from groups of springs in three different localities, which afford an approximate mean daily yield of 418,500 gallons by gravity. In addition, there are three other sources from which potable water is pumped, the mean daily yield being about 693,000 gallons. Besides the drinking water there is a brackish water found at Armier, and pumped to Valetta, where it is used for watering streets and flushing sewers. The main storage reservoirs for drinking water are at Ta Kali, near Attard, and are capable of holding 16,865,200 gallons. The total storage capacity of the island is 18,980,000 gallons. The gathering grounds for the water supply are for the main part in thinly populated portions of the island. The water is collected in galleries driven in the rock deep below the surface, and conveyed by iron pipes to the tank or pumping station. From there it is distributed by cast-iron pipes or by stone channels built in, and lined with cement. The water is usually distributed by means of stand-pipes in the villages. In Valetta the houses generally have taps, but they are often without them, and outside the area surrounding the harbours taps are seldom found in the houses. In some villages there are large underground tanks provided by the roadside, which are filled in the winter from the public water supply. These tanks are seldom fitted with pumps, and in consequence become very foul from the constant lowering into them of buckets which have been allowed to stand on the roadside. The vicinity of pervious cesspools provides a possibility of pollution which is often present.

Practically every house in Malta has underneath it a large tank for collecting rain-water from the roof. This water is generally preferred to the public water supply for drinking, possibly because it is cooler

in summer. The contents of these tanks very often show signs of pollution, nor can this be wondered at when it is considered that the roof is very much used by the family in summer, and by their cats and dogs. Tanks are never supplied with pumps, and the bucket which is lowered into them by a rope is often placed on the yard flags amidst slop water and pollutions due to animals or to the neighbouring water-closet. There are very few wells in Malta.

In Gozo the public water supply is obtained from similar sources to that of Malta, the total mean daily supply amounting to about 143,000 gallons, derived from three principal sources. About 90,000 gallons of this water, coming from two sources, is brackish, and about 50,000 gallons, coming from the other source, is good water. The two qualities are mixed before distribution, and the result is a water containing considerable quantities of magnesium salts, which is liable to affect new comers prejudicially.

In the villages of Xahra and Nadur there are wells, a few also being found in Victoria; but elsewhere the usual rain-water tank system prevails, and is liable to the same dangers as in Malta.

All the villages in Gozo except Xahra, Zebbug, Nadur, and Kala have the public water supply, and Nadur is about to be supplied.

Hospitals.—There is a hospital for infectious diseases in connection with the Lazaretto on Manoel Island. It is intended for the isolation of small-pox, scarlet fever, diphtheria, and erysipelas. The buildings are out of date.

The Central General Hospital is at Floriana. It has 226 beds, including the Seamen's Hospital, which adjoins it. It receives cases of enteric and Mediterranean Fever, besides surgical and other cases. No attempt is made to isolate the Mediterranean Fever cases from the others. The methods of this hospital in the matter of cleansing the patient and disposal of the contents of bed-pans of enteric and Mediterranean patients are unsatisfactory. Reference will be made to this matter later. There is no proper hospital sink, and the laundry is inadequate. All infected clothing is despatched to the poor-house laundry. It is said to be steeped in corrosive sublimate solution before being sent, but I saw no signs of the process at my visit, except some barrels containing water, which were shown me as the receptacles used for steeping. There is a general hospital at Citta Vecchia, known as the Santo Spirito Hospital, containing some 70 beds, and there is a similar institution in Victoria, Gozo, with about 60 beds.

The quarantine hospital on the island of Comino is well isolated. It is intended only for ship-borne cholera, yellow fever, or plague.

There is a large building at Marfa, well isolated, which is intended to serve for cases of exotic disease amongst the inhabitants of Malta.

The military hospitals are seven, six in Malta and one in Gozo. The Station Hospital, Valetta, accommodating about 200 patients, is the

ancient hospital of the Knights of St. John. The building is unsuitable for a modern hospital. It contains no hospital sink. Attached to it is the hospital of the Royal Malta Artillery.

The Royal Naval Hospital at Bighi contains about 200 beds.

Sanitary Administration.—There is a Council of Health, consisting of twenty members, six of whom are medical men. It is their duty to advise the Government on sanitary regulations, on quarantine measures, on public works in connection with hygiene, including drainage and water supply, and on all other matters of public health. The Council has power to suggest measures, inquiries, and scientific investigations in connection with public health. The Council meets every two months.

The public health department is directed by the Superintendent of Public Health, Mr. R. P. Samut, M.R.C.S. Eng., who receives £400 per annum. It is his duty to watch over and direct the medical officers of health, the sanitary inspectors, and all other officers of the department, and to advise them. He has also to inspect at intervals the hospitals, quarantine establishments, slaughter-houses, charitable institutions, prisons, etc. Finally, he has to advise the Governor, when required, on public health questions, and he has to draw up an annual report on the sanitary state of the islands, and send it to the Governor.

The Superintendent of Public Health thus reports direct to the Governor, and the functions of the Council of Health are purely advisory, or at most suggestive. The Superintendent solely is responsible for the annual report. His position is one of great responsibility, demanding experience and a high degree of expert knowledge.

There are two medical officers of health for Malta—Dr. Caruana Xicluna, who receives £350 a year, and Dr. F. Xuereb, who receives £250. These gentlemen divide their duties—the first-named superintending buildings, zymotic diseases, foods, shops, and noxious trades, while the latter looks after drainage, notifications, isolation, disinfection, and overcrowding.

There is a medical officer of health for Gozo—Dr. E. Calleja, who receives £120 a year. The Sanitary Engineer, Mr. C. Mallia, receives £170, with £30 additional as superintendent of drains. There are 18 sanitary inspectors in Malta, receiving about £60 a year each. In Gozo there are four, who receive salaries on the same scale. There is an inspector of markets, who receives £110 a year; Mr. MacFarlane, M.R.C.V.S., who superintends the slaughter-house, receiving £30 a year.

There are 22 district medical officers in Malta, and four in Gozo. Up to 1885 these officers received £10 a year for certain public health duties; but since then each of them receives a lump sum varying from £60 a year to £140 a year to cover all their duties, which are in the main the same as those of our poor-law district medical officers. They

also perform vaccination twice a year free of cost. Their public health duties are to inspect infected premises, and determine whether the patient must be removed to hospital or not ; to inspect bad food if called in by a sanitary inspector, and to inspect midwives once a month, and see that their equipment is adequate and clean.

The district medical officers are under the control of the Comptroller of Charitable Institutions, as are also the hospitals and other charitable institutions.

There is a public analyst and bacteriologist, with two assistants. Dr. T. Zammit has held the post since January, 1891.

The annual report of the public health department has of late years been very disappointing. Formerly it contained comments and suggestions, but it is now merely statistical and conveys little information as to the conditions which exist and have to be dealt with by the department.

Some of the sanitary inspectors are hard-working, intelligent men, who know their districts ; but not a few are entirely ignorant of the elements of sanitation, and in some cases even of the conditions prevailing in their districts. Close supervision and drastic weeding out is required amongst them. The Government have made a new departure this year in sending three young men to England to be trained as sanitary inspectors. They are to be followed by others if the experiment prove successful. I have great hopes that it will. Some of the sanitary inspectors make house-to-house inspections daily, but others never do so unless a case of infectious disease arise. The poorer people are too ignorant of sanitation to make complaint, so that without frequent inspection grave conditions may be allowed to exist for long periods. The public health department have issued a general order to the sanitary inspectors not to report faults in drainage unless urgent, on the grounds, I was informed, that all the villages would some day be sewered. The order is liberally interpreted by many of the inspectors, and was quoted to me in extenuation of such conditions as cesspits ventilated into living rooms, sewers ventilated into houses by unsealed water-closets, leaky cesspits, etc.

There is a sanitary commission appointed by the Governor for the maintenance and construction of drainage. The Superintendent of Public Works is chairman, and there are six other members, four of whom are medical men.

Notification of Infectious Disease.—There is no payment for notification, and though there is a penalty for neglect to notify, it is difficult to exact, and in point of fact never has been exacted. Many considerations interfere with the accurate notification of Mediterranean Fever. For instance, persons who die of it are not permitted burial in a church, a cherished privilege outside the fortified towns, or a private practitioner wishes to spare his patient the annoyance and

expense of lime-washing and disinfection. In addition, the diagnosis is often difficult, the serum test not generally being applied. The consequence is, only severe cases are notified, and not always these. In the official record of the notifications, the age and sex of the patient is generally unrecorded; in many instances even the name is not recorded, nor the number of the house.

A Maltese medical man of experience told me he did not think more than a third of the cases of Mediterranean Fever that occurred in Malta were notified, and not more than a fifth of those in Gozo. I think this estimate is not far wrong. In addition, I found that many English cases attended by Army doctors were not notified.

The following diseases are notifiable:—Plague, cholera, yellow fever, small-pox, scarlet fever, diphtheria, diphtheritic croup, typhus fever, enteric fever, measles, remittent fever (Mediterranean), febrile puerperal diseases, continued fever (on 7th day), erysipelas, epidemic spinal meningitis, chicken-pox, influenza, whooping cough.

Disinfection.—The usual means adopted are fumigation by burning sulphur, soaking washable materials in corrosive sublimate solution, and lime-washing. Bedding and other articles unsuitable for soaking are not sent to the steam disinfecter in cases of Mediterranean Fever, seldom indeed in any disease.

Isolation.—Small-pox and diphtheria are isolated, and the early cases in outbreaks of measles or scarlet fever. The routine adopted in these diseases is as follows:—The case is visited by the District Medical Officer, who reports whether it can be isolated at home or not. If it can be isolated at home, a man is sent to act as health guard, whose business it is to prevent communication between the sick room and the public. If the case has to be removed, a police sergeant is sent with the ambulance.

Mediterranean Fever is not isolated.

Sanitary Law is embodied in ordinances enacted by the Governor with the advice and consent of the Council of Government. They cover much the same ground as our own public health laws, though some of the Maltese ordinances are more stringent. They include regulations as to noxious trades, bake-houses, milk-shops,* buildings, markets, refuse in streets, etc.†

There is a quarantine medical officer, with three assistant medical officers, and a veterinary surgeon.

* The usual source of milk in Malta is the goat. These animals are driven about the streets in flocks, and are milked at the customer's door into his own vessel. The udders, which are abnormally large, often touching the ground, are very liable to be soiled. The proprietors of herds are so many that it is always difficult to ascertain from a householder where he has got his milk. No regulations are in force for the effectual control of these vendors.

† The regulation against throwing refuse and offal into the streets is not enforced, or very feebly so.

PART II.—MEDITERRANEAN FEVER.

Introductory.—I do not propose in this report to deal with the history and literature of Mediterranean Fever, or with its symptoms, treatment, or distribution outside Malta, except very briefly, and in so far as these have a direct bearing upon my own part of the work of the Commission.

The study of Mediterranean Fever, virtually commenced by Marston's paper in 1861, received its great impetus from the discovery of the *Micrococcus melitensis* by Bruce in 1887. After this, for many years the difficulty of diagnosis caused by the strong resemblance between this fever and other diseases endemic in Malta, such as enteric fever and the fever known as "simple continued," retarded investigation and detracted from the value of the figures recorded.

In 1897, Wright and Semple, by introducing the serum agglutination test, placed matters on a more exact basis. This method was in 1900 adopted as a routine practice in the Army, and shortly after in the Navy.

Since the publication of Hughes' book in 1897, Mediterranean Fever has attracted considerable attention in the Army and Navy, and much has been written about it. With the exception of Zammit's paper in 1902, little appears to have been done in the way of studying its behaviour amongst the civil population of Malta, either by the local medical men or by others. It was partly for this reason that I devoted most of my attention, during my stay in Malta, to the disease as it occurred amongst the Maltese.

From the outset I found myself confronted by two difficulties, the first, a badly administered system of notification, which has been already referred to in Part I of this report, and closely allied with it considerable unreliability of diagnosis, due partly to the fact that the serum agglutination test is not generally in use in Malta outside the Army and Navy, in spite of the facilities afforded by the Bacteriological Laboratory at Valetta. My second difficulty was the fact that very few Maltese speak English, and that their natural politeness leads them generally to try and give the reply they deem most likely to please, and not that which is most strictly in accordance with the facts. Add to this a strong distrust of the sanitary authority, whose objects they are in general unable to understand, and whose visits they regard solely as a probable source of expense to themselves in the way of white-washing or cleansing, and it will be understood that accurate information was not always easy to come by.

The Geographical Distribution of Malta Fever will be of importance in the future study of the conditions which favour the spread of the disease.

The following list of places, from which Mediterranean Fever has been reported, is taken, with slight additions, from the "Journal of the R.A.M.C.," vol. ii, No. 4 :—*Spain*—Gibraltar; *Islands of the Mediterranean*—Balearic Islands, Corsica, Sardinia, Sicily, Malta, Gozo, Cyprus, Crete; *Italy*—Rome, Naples, Caserta, Benevento, Campobosso, Aricca, Terano, Fermo, Padua, Cittanova, etc.; *Greece*—Athens, Cephalonia; *Turkey*—Constantinople, Smyrna; *Palestine*—Jerusalem; *Africa*—Tunis, Algiers, Alexandria, Suakin, Massowah, Zanzibar, Kimberley (?); Aden; *India*—Calcutta, Mian-Mir, Nowshera, Secunderabad, Simla, Delhi, Lucknow, Agra, Allahabad, Choabattia, Subatha, Assam, Swat Valley; *China*—Hong-Kong, Philippine Islands, Fiji Islands; *North America*—Mississippi Valley; *West Indies*—Cuba, Puerto Rico; *South America*—Venezuela, Brazil, Montevideo.

This list will probably undergo considerable enlargement and alteration in the future, and I will only say in connection with it that there are factors which I am not in a position to take into account, such as the reliability of the diagnosis or of the cultures by means of which the diagnosis has been confirmed, and the fixing of the place where infection took place, having regard to the prolonged liability to relapse.

Hitherto, Mediterranean Fever has not been reported north of the 45th parallel or south of the 40th.

Incubation Period.—The general impression amongst Maltese medical men seems to be that the usual incubation period of Mediterranean Fever is not more than 8 or 10 days.

The following cases have occurred in the course of laboratory work with the *Micrococcus melitensis* in places where there was no prevalence of Mediterranean Fever and no apparent source of infection other than in relation with infective material in the laboratory :—

| | Incubation period. |
|---|-----------------------|
| 1.—S. From an accidental prick with a syringe needle which contained a living culture | 15 days |
| 2.—W. From purposeful hypodermic injection of a living culture | 16 " |
| 3.—B.S. From accidentally drawing into the mouth a small quantity of living culture through the mouth..... | 8 " |
| 4.—E. From the same kind of accident as 3 | 6 " |
| 5.—S. From accidental wound of the conjunctiva with a portion of a broken tube which had contained living culture*..... | 5 " |

Besides the above, Fleet-Surgeon Bassett-Smith has informed me of

* In this case, examination by an oculist, soon after the breakage of the tube, failed to disclose any wound of the conjunctiva.

another laboratory case in which the occasion of inoculation could not be traced.

The five cases given above are too few to afford sufficient basis for trustworthy induction. They are, moreover, open to the objection that infection did not necessarily take place on the occasions cited, but may have occurred at some other time in the course of work with infective material. But after due allowance made for the latter consideration, No. 2 is to be regarded as of materially greater value as a guide to the incubation period of Mediterranean Fever than any of the others, since, in this instance, there was a definite and purposeful introduction into the system of a presumably sufficient amount of living culture of *Micrococcus melitensis*, accompanied by record of the time of such introduction, and by subsequent outlook for the first appearance of illness. The remaining four cases, which are marked by considerable diversity in respect of the incubation periods inferred, are more open to challenge, and, therefore, afford less trustworthy guidance in this matter than does No. 2.

I have made inquiry with a view to finding how many cases of Mediterranean Fever have been observed to occur on board our ships of war after leaving a Mediterranean port and before touching at another Mediterranean port.

Fleet-Surgeon Bassett-Smith has kindly examined his records and sent me 13 cases which occurred on board ship, but none in which it could be confidently said that the fever occurred more than 14 days after leaving the last Mediterranean port of call. He included three cases in which Mediterranean Fever occurred three weeks after the ship left Malta, but he was unable to say she had not subsequently called at a Mediterranean port before the onset of the fever. He also included a case in which Mediterranean Fever occurred 12 months after leaving the Mediterranean, but said that the patient had a slight attack of fever while on the Mediterranean station, so that the possibility of a relapse cannot be excluded.

Fleet-Surgeon Bassett-Smith also sent me the following remarkable case:—A. B. left Gibraltar December 20, 1903, having had no attack of fever at the time and feeling quite well. He arrived home for Christmas, went on leave for a month, and then took on duty attached to the "Excellent" gunnery establishment at Gosport. About February 20, 1904, acute fever set in, for which he was sent to the Royal Naval Hospital, Haslar, as a case of enteric fever. His blood was examined in the first fortnight, and gave a negative reaction for enteric, but a positive for Mediterranean, and later on the *Micrococcus melitensis* was isolated from his blood. This case must have had an incubation period of two months if it be conceded that infection occurred in the Mediterranean. There is the possibility that he was infected from some unrecognised case at Gosport, but this possibility

is largely discounted by the fact that many cases of Mediterranean Fever are annually treated at the Royal Naval Hospital, Haslar, without any recorded spread of infection. The case, though exceedingly interesting, will be of greater value in the consideration of the incubation period of Mediterranean Fever, should other like cases occur hereafter ; at present it stands alone.

From Staff-Surgeon Gilmour I received record of eight cases which occurred on warships at sea, and two others I saw myself in Malta, but none of them occurred more than 14 days after the ship had touched at a Mediterranean port.

Ships while on the Mediterranean station are seldom or never more than a few days without touching at a port, so that their records are unlikely to afford any guidance until they leave the station for home.

I received from Lieutenant-Colonel Rhodes, R.A.M.C., details of a search which he caused to be made, at my request, in the records of the R.A.M.C. at Malta for the period January 1, 1901, to August 31, 1904. During that time only two cases could be found in which Mediterranean Fever had occurred less than 14 days after the patient's arrival in Malta. The first case was a Munster Fusilier, who was admitted to hospital suffering from Mediterranean Fever on February 22, 1901, eight days after the regiment's arrival in Malta, from England. The second case occurred in the Sussex Regiment, and was admitted to hospital on July 7, 1904, 11 days after the regiment's arrival in Malta, from England.

Further evidence is required before a definite average incubation period can be established. It may, however, be provisionally stated that the data available tend, in some degree, to suggest that the incubation of Mediterranean Fever ranges about a period of 14 days.*

Distribution of Mediterranean Fever in the Maltese Islands.

Mediterranean Fever occurs in every part of Malta and Gozo, on the sea coast and inland, though as a rule its relative incidence is less severe in the villages more remote from the capitals.

The figures given below with regard to cases have been abstracted from the civil official notification returns, where Mediterranean Fever is generally notified under the name of remittent fever. There are in these returns numerous cases notified under the name continuous fever. These Zammit has included in his returns as Mediterranean Fever cases. His grounds are that in Malta all fevers lasting more than a week are notifiable by law, and since all the named fevers—

* Bruce says that cases have occurred in as short a time as 6 days after arrival in Malta. Hughes, while stating his belief that the incubation period is sometimes as short as 3 days, considered that 10 to 15 days is the most usual time.

such as enteric, etc., are notified separately under their proper headings, the residue returned as continuous fever are in reality Mediterranean Fever. He does not, however, reckon with the fever known as "simple continued fever," the most common form of illness in Malta during the hot weather, or else he does not consider that simple continued fever commonly lasts more than a week.

I have examined the records of the naval hospital in Malta, where the diagnosis is at least as careful as in Malta generally, and I find that during the five years 1897 to 1901 the average duration of cases returned under the heading "Other Continuous Fevers" is over eight days. Practically, all these cases must have been "simple continued fever," since Mediterranean Fever, enteric, malaria, and other named fevers are placed under separate headings. I think it, then, more probable that the majority of the cases noted in the Maltese notification returns as continuous fever were cases of "simple continued fever" than that they were cases of Mediterranean Fever. I have, therefore, included in tables dealing with cases of Mediterranean Fever only those cases which were entered in the Maltese notification records as remittent fever, or as Mediterranean Fever.

Table I (pp. 18 and 19) shows the number of cases of Mediterranean Fever which occurred in each of the districts of Malta and Gozo amongst the civil population during each year of the period 1894 to 1903, together with the mean estimated population of each district during the same period, the average number of cases of Mediterranean Fever per 10,000 of population per year, and the number of deaths from Mediterranean Fever during the whole period.

The very general distribution of Mediterranean Fever throughout Malta is perhaps the most striking feature of this table. It will be seen, too, that it is by no means the localities closest to the harbours which suffer the most severely. Hamrun, a somewhat squalid suburb, and the combined villages of Lia, Attard, and Balzan show the heaviest incidence, while Valetta and the three fortified towns, Cospicua, Senglea, and Vittoriosa, are amongst the least severely attacked. In some respects, however, the latter four places cannot fairly be compared with the remainder of the island. All four towns are paved, drained, and scavenged, a state of affairs not found in any other part of the island.*

Disregarding Valetta and the three fortified towns above mentioned, it will be found that the severity of incidence in Malta depends roughly on the amount of the population. The average incidence throughout

* Floriana is drained but not paved, and the scavenging is not so well carried out as in Valetta; moreover, a vast number of country people pass through it each morning to reach Valetta, and complaints have been made that they use the fortifications around Floriana as a latrine. Curmi was sewered in 1901, Sliema in 1902, and Misida and St. Julians in 1903.

Malta as a whole, is 32 cases per year per 10,000 of population, and the average for places other than the four towns first mentioned is 37·8, neglecting the cases which occurred in public institutions. The average number of inhabitants for each of these places is 4240. Twelve of them contain more than 4240 persons each, and in 8 of the 12 the average incidence is greater than 37·8. Fourteen districts have less than 4240 inhabitants, and in 12 of them the average incidence is less than 37·8 per 10,000. The mean incidence on the group with over 4240 population is 41·6 per year per 10,000 during the 10-year period, while that upon the group with less than 4240 population was for the same period 26·7 per year per 10,000. This is no doubt a very rough classification, and there are notable exceptions, such as Curmi. On the other hand, the notification returns dealt with are not sufficiently reliable to justify any but the most general groupings, perhaps not even these. The classification, such as it is, would tend to show that, outside Valetta and the towns named, the greater the aggregation of inhabitants living in one locality, the greater is the proportional number of cases of Mediterranean Fever that occurs amongst them. The returns from Gozo do not show the same result, but then the numbers dealt with are smaller and the returns themselves probably more inaccurate than in Malta.

Density of population upon area outside Valetta, Cospicua, Vittoriosa, and Senglea appears to have some influence on the incidence of Mediterranean Fever. Floriana, Hamrun, Misida and Pietá, Sliema, Zeitun, and Mellieha are the most densely populated, all having more than 100,000 persons to the square mile. With the exception of Mellieha, and in a lesser degree Zeitun, these places all have a case incidence above the average. On the other hand Zebbug, Sigguei, Axiak, Gudia, Chircop, Safi, Zurrigo, and Krendi are the least densely populated, each having less than 30,000 persons per square mile, and the proportional incidence on all these places, except Zebbug, is less than the average.*

For purposes of comparison, it has been customary for some years past to divide Malta into three areas—(1) *an urban drained area*, comprising Valetta, Floriana, Cospicua, Vittoriosa, and Senglea; (2) *a suburban undrained area*, comprising Misida, Pietá, Sliema, St. Julian's, Hamrun, Birchirchara, Curmi, Zabbar, Tarxien, and Paola; and (3) *a rural area*. Comparing the three areas for the period 1894 to 1903, it appears that the average number of cases of Mediterranean Fever per year per 10,000 inhabitants was—

| | |
|-----------------------------------|------|
| (1) Urban drained area | 18·8 |
| (2) Suburban undrained area | 41·8 |
| (3) Rural area | 33·4 |

* It will be seen later, when considering the maps, that density of population upon area in parts of districts, does not show the same influence.

Table I.

| | Number of cases of Mediterranean Fever notified. | | | | | | | | | | Mean estimated population during the period 1894-1903. | Average number of cases of Mediterranean Fever per year per 10,000 of mean estimated population during the period 1894-1903. | Number of deaths from Mediterranean Fever during the period 1894-1903. |
|-------------------------------|--|-------|-------|-------|-------|-------|-------|-------|-------|-------|--|--|--|
| | 1894. | 1895. | 1896. | 1897. | 1898. | 1899. | 1900. | 1901. | 1902. | 1903. | Total. | | |
| Malta— | | | | | | | | | | | | | |
| Valetta | 17 | 16 | 52 | 22 | 25 | 39 | 44 | 49 | 46 | 30 | 340 | 14.3 | 53 |
| Floriana | 16 | 7 | 34 | 28 | 18 | 43 | 31 | 28 | 27 | 38 | 270 | 45.7 | 20 |
| Misda and Pietà | 5 | 1 | 3 | 20 | 19 | 26 | 21 | 24 | 60 | 29 | 208 | 57.1 | 6 |
| Sienna and St. Julian's | 44 | 24 | 53 | 57 | 33 | 55 | 43 | 42 | 67 | 52 | 470 | 43.3 | 39 |
| Hamrun | 4 | 38 | 71 | 86 | 73 | 92 | 81 | 104 | 34 | 24 | 607 | 10,856 | 26 |
| Cospicua | 6 | 3 | 74 | 27 | 12 | 16 | 9 | 21 | 8 | 25 | 201 | 9,364 | 31 |
| Vittoriosa | 1 | 6 | 7 | 13 | 37 | 33 | 26 | 14 | 13 | 14 | 164 | 12,128 | 16 |
| Senglea | — | 1 | 30 | 14 | 11 | 7 | 2 | 4 | 8 | 7 | 84 | 7,162 | 31 |
| Notabile and Rabato | 14 | 5 | 8 | 17 | 32 | 76 | 14 | 16 | 36 | 25 | 243 | 8,012 | 10 |
| Dingli | — | — | — | — | — | 4 | 7 | 3 | 16 | — | 90 | 8,045 | 36 |
| Zebbug | 3 | 24 | 53 | 17 | 18 | 13 | 68 | 19 | 28 | 47 | 290 | 39.5 | 2 |
| Siggien | 2 | — | 4 | 10 | 16 | 33 | 8 | 3 | 6 | 4 | 86 | 53.5 | 21 |
| Birchirchara | 10 | 28 | 30 | 25 | 37 | 103 | 67 | 58 | 41 | 42 | 441 | 26.9 | 9 |
| Lia, Attard, and Balzan | 38 | 27 | 53 | 19 | 12 | 52 | 25 | 35 | 31 | 33 | 330 | 54.2 | 42 |
| | | | | | | | | | | | | 73.0 | 20 |

| | | | | | | | | | | | | | |
|------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|---------|------|-----|
| Naxaro | 6 | 6 | 15 | 4 | 16 | 11 | 21 | 10 | 14 | 109 | 3,443 | 31.7 | 6 |
| Musta | 5 | 12 | 8 | 32 | 27 | 34 | 24 | 27 | 24 | 191 | 4,675 | 40.9 | 27 |
| Gargur | — | — | 10 | — | 3 | 7 | 1 | 6 | 4 | 31 | 1,355 | 22.9 | 5 |
| Melleha | — | 3 | — | 5 | 2 | 7 | 5 | 3 | 1 | 26 | 2,233 | 11.6 | 4 |
| Curni | 2 | 1 | — | 4 | 2 | 5 | 17 | 8 | 6 | 48 | 7,994 | 6.0 | 9 |
| Luca | — | 1 | — | 2 | — | 3 | 2 | 6 | 12 | 26 | 3,318 | 7.8 | 5 |
| Tarxien and Paola .. | 6 | 7 | 8 | 5 | 16 | 3 | 2 | 24 | 17 | 101 | 4,476 | 22.6 | 15 |
| Zurrico | 6 | 3 | 11 | 12 | 22 | 8 | 4 | 5 | 3 | 87 | 3,589 | 24.2 | 8 |
| Saf | — | 2 | 2 | — | 2 | — | 1 | 2 | 2 | 11 | 350 | 31.4 | 1 |
| Krendi | 1 | 7 | 7 | 6 | 5 | 1 | 1 | 1 | — | 31 | 1,559 | 19.9 | 5 |
| Micabiba | 1 | 10 | 3 | 2 | 2 | — | — | 2 | 2 | 23 | 1,192 | 19.3 | 1 |
| Chircop | — | 2 | — | 3 | 1 | 1 | 1 | 1 | 2 | 14 | 619 | 22.6 | 1 |
| Zeitun | 55 | 14 | 44 | 10 | 9 | 17 | 14 | 7 | 22 | 222 | 7,492 | 29.8 | 12 |
| Zabbar | 47 | 7 | 18 | 17 | 18 | 14 | 24 | 13 | 27 | 214 | 5,549 | 38.6 | 20 |
| Axjak | 9 | 3 | 1 | 2 | 1 | 4 | 6 | 1 | 1 | 33 | 1,493 | 23.1 | 1 |
| Gudia | 3 | 2 | 3 | 2 | 3 | 4 | — | — | 4 | 30 | 1,141 | 26.3 | 1 |
| Public institutions... | 9 | 15 | 13 | 6 | 27 | 43 | 23 | 14 | 7 | 173 | | | |
| Total | 310 | 247 | 495 | 455 | 748 | 608 | 572 | 551 | 518 | 5134 | 160,603 | 32.0 | 452 |
| Gozo— | | | | | | | | | | | | | |
| Victoria..... | 4 | 11 | 4 | 9 | 10 | 2 | 6 | — | 13 | 71 | 6,101 | 11.1 | 3 |
| Garbo | — | — | — | — | 1 | 3 | — | — | — | 5 | 1,708 | 2.9 | 5 |
| Zebbug | — | — | 2 | 7 | 7 | — | — | 1 | 1 | 25 | 1,166 | 4.3 | — |
| Sannat..... | — | — | — | 1 | — | 8 | — | — | 1 | 146 | 1,098 | 22.9 | 4 |
| Xahra | 3 | 2 | 51 | 28 | 18 | 22 | 13 | 5 | 2 | 43 | 2,509 | 58.3 | 7 |
| Xeuchia | — | — | 10 | 6 | 2 | 2 | 7 | 7 | 9 | 53 | 1,718 | 25.0 | 2 |
| Nadur and Kala | 2 | 1 | 1 | — | 3 | 1 | 24 | 14 | 7 | 18 | 4,079 | 13.0 | 7 |
| Ghainstelem..... | — | — | — | 1 | 1 | — | 3 | 10 | 1 | 9 | 1,106 | 16.3 | 2 |
| Ospizio | — | 1 | — | — | 5 | 2 | — | — | 1 | | | | |
| Total | 9 | 5 | 68 | 52 | 49 | 40 | 53 | 37 | 35 | 375 | 19,480 | 19.2 | 30 |

Considerably below the average number of cases occur in the urban area, and considerably above the average in the suburban area, while the rural area suffers in much the same degree as Malta taken as a whole.

The urban area is better drained and paved, scavenging is more efficiently carried out, and it has more public conveniences than other parts of the island. On the other hand, there is in the urban area equal or greater aggregation of persons in one locality, and more overcrowding upon area. There is no marked difference between the urban and suburban areas as a whole in the matter of the wealth and station of their inhabitants. Parts of Valetta such as the Manderaggio contain the very poorest of the population, and these parts do not appear to suffer out of proportion to their numbers. The three cities also have many very poor inhabitants; but Sliema and St. Julian's are probably the most wealthy and fashionable parts of the island.

There are some stumbling blocks in the way of all attempts to generalise from the table above. The most striking is the difference in the incidence upon Curmi and upon Birchirchara. These two towns, for they are really small towns rather than villages, are situated close together, on the same kind of soil, with the exception that a part of Curmi is on alluvial soil. They contain about the same number of inhabitants, and the same kind of houses, any difference being that there are more good houses in Birchirchara, yet the incidence of Mediterranean Fever upon the latter has been nine-fold as great as upon Curmi during the period 1894 to 1903. I have examined these villages from many points of view, but I have hitherto found nothing that would account to my mind for the difference. It may be that the personal factor is of unusual weight here in the matter of notification. I hope, however, to have some more facts to consider in this connection when the returns which are now being collected in Malta are due for examination.

I prepared a map for the purpose of studying the distribution of Mediterranean Fever during the period January 1, 1899 to July 31, 1904, in Valetta and Floriana respectively.

In Valetta there is no marked aggregation of cases in proportion to population. The apparent aggregations of cases in the Manderaggio, in Strada S. Giuseppe, and in Str. Pozzi are due simply to the fact that these localities are very thickly populated, consisting mainly of houses each of which contains many families—"Kerreyas" or common lodging-houses. I was informed, for instance, that about 3000 people live in the Manderaggio.

In Floriana, also, most of the groupings of cases are due to cases occurring in different families living in the same "Kerreya." There is, however, a remarkable immunity from attack noticeable in Piazza

Maggiore, Piazza Britannica, and Strada Giardino, all of which face on to broad open spaces, and consist in the main of better class houses. This may be partly explained by the fact that the English doctors do not as a rule comply with the notification law. Most better class streets in Floriana, Sliema, and St. Julian's are occupied to a considerable extent by English people, who are generally attended by English doctors.

Another map showed the distribution of Mediterranean Fever in Hamrun during the period January 1, 1892 to July 31, 1904. Here there are not so many "Kerreyas," and in consequence there is less apparent grouping of cases than in Valetta and Floriana.

A third map showed the distribution of cases during the same period in Sliema. Mediterranean Fever cases here show a distinct preference for the southerly slope towards Sliema Creek as opposed to the northern slope towards the open sea, and the houses in Strada It-Torri, which face the sea, seem to have escaped attack. But many of these houses are occupied by English people, and in any case the streets running down to Sliema Creek are much more densely inhabited.

My maps of Misida and Pietá proved valueless, because the notification returns left so many houses unindicated, the names of the streets only being given.

The other maps in my possession serve only to show the same lack of definite aggregation of cases around a particular centre.

The following table shows the distribution of Mediterranean Fever during the period 1901 to 1903 in the Mediterranean Fleet, including such of His Majesty's ships as have called at Malta in passing through the Mediterranean Sea during that period. (Table II.)

Many of the cases occurred on board ships at considerable intervals of time from the vessel's last call at Malta, and it seemed useful to attempt some discrimination between cases that were possibly infected at Malta and those probably infected in some other port. I have accordingly separated the cases which occurred between 6 days and 20 days after a ship's last visit to Malta, and placed them in a separate column headed "Cases connected with Malta." This admits a certain amount of error, because a ship may have visited other ports and become infected at them during the interval of 20 days. I have no record of the ports visited other than Malta. In a few cases no date was assigned for the onset of the fever, and these I have put in the column referred to, in proportion to the number of days in the year spent at Malta by the ship on which they occurred.

For the three years set out in the table the ships, as a whole, show an incidence of 28.55 per 1000 of strength constantly in Malta, a rate corresponding closely with that of the garrison in Malta during the same three years (28.08 per 1000). The two rates, however, are not really comparable, that of the Navy being calculated on a population

| Name of Ship. | 1901. | | | | | 1902. | | |
|----------------------|------------------|--------------------------------|---|--|---------------------------|------------------|--------------------------------|---|
| | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever con- nected with Malta. | Men. Days in Malta. | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. |
| H.M.S.— | | | | | | | | |
| Renown..... | 758 | 255 | 16 | 14 | 198,290 | 758 | 166 | 17 |
| Ramillies | 746 | 158 | 34 | 30 | 117,868 | 746 | 123 | 7 |
| Cæsar | 759 | 211 | 13 | 11 | 160,149 | 759 | 190 | 19 |
| Illustrious | 759 | 262 | 16 | 15 | 198,858 | 759 | 161 | 12 |
| Victorious | 759 | 133 | 14 | 10 | 100,947 | 759 | 170 | 6 |
| Royal Oak | 714 | 204 | 4 | 2 | 147,696 | 714 | 60 | 4 |
| Royal Sovereign | 714 | 254 | 19 | 18 | 180,356 | 714 | 97 | 8 |
| Empress of India.... | 714 | 104 | 9 | 8 | 74,256 | — | — | — |
| Canopus | 753 | 219 | 16 | 12 | 164,907 | 753 | 209 | 21 |
| Theseus..... | 546 | 173 | 10 | 8 | 57,158 | 546 | 67 | 12 |
| Andromeda | 679 | 209 | 8 | 5 | 141,911 | 679 | 142 | 6 |
| Vindictive..... | 429 | 183 | 1 | 0 | 78,507 | 429 | 162 | 6 |
| Tyne | 101 | 266 | 1 | 1 | 26,866 | 101 | 231 | 3 |
| Hibernia..... | 676 | 365 | 14 | 14 | 246,740 | 729 | 365 | 20 |
| Devastation | 410 | 156 | 1 | 0 | 63,960 | 410 | 5 | 0 |
| Rupert | 294 | 19 | 13 | 4 | 5,586 | 294 | 10 | 0 |
| Diana | 450 | 116 | 7 | 5 | 52,200 | 450 | 193 | 16 |
| Hood..... | 694 | 174 | 7 | 7 | 119,756 | 694 | 174 | 21 |
| Harrier..... | 122 | 124 | 0 | 0 | 15,128 | 124 | 55 | 0 |
| Vulcan | 443 | 188 | 5 | 3 | 61,184 | 443 | 230 | 8 |
| Pegasus..... | 226 | 95 | 3 | 2 | 21,470 | 226 | 117 | 4 |
| Implacable..... | 780 | 31 | 3 | 0 | 24,180 | 780 | 213 | 25 |
| Gladiator | 429 | 188 | 4 | 2 | 59,202 | 429 | 216 | 6 |
| Scout..... | 147 | 72 | 1 | 1 | 10,584 | — | — | — |
| Pioneer..... | 224 | 172 | 1 | 0 | 38,528 | 224 | 209 | 9 |
| Barham..... | 175 | 187 | 2 | 1 | 32,725 | 175 | 194 | 1 |
| Pyramus..... | 224 | 194 | 9 | 8 | 43,456 | 224 | 178 | 12 |
| Surprise | 107 | 255 | 9 | 9 | 27,285 | 107 | 258 | 4 |
| Halcyon | 122 | 16 | 0 | 0 | 1,952 | — | — | — |
| Dryad..... | 121 | 212 | 6 | 5 | 16,552 | 121 | 226 | 7 |
| Hussar | 121 | 154 | 0 | 0 | 18,634 | 121 | 176 | 1 |
| Salamander | 91 | 134 | 0 | 0 | 12,194 | — | — | — |
| Speedy | 91 | 192 | 3 | 3 | 17,472 | 91 | 246 | 6 |
| Ardent | 53 | 217 | 0 | 0 | 11,501 | 53 | 169 | 0 |
| Dragon | 53 | 262 | 0 | 0 | 13,886 | 53 | 146 | 0 |
| Kangaroo | 63 | 61 | 0 | 0 | 3,843 | 63 | 237 | 0 |
| Desperate | 63 | 27 | 0 | 0 | 1,701 | 63 | 276 | 0 |
| Myrmidon | 63 | 27 | 0 | 0 | 1,701 | 63 | 242 | 0 |
| Chamois | 63 | 56 | 0 | 0 | 3,528 | 63 | 276 | 0 |
| Bruizer..... | 53 | 227 | 0 | 0 | 12,081 | 53 | 140 | 0 |
| Banshee | 53 | 227 | 0 | 0 | 12,031 | 53 | 290 | 0 |
| Foam | 63 | 260 | 0 | 0 | 16,380 | 63 | 233 | 0 |
| Earnest..... | 63 | 274 | 0 | 0 | 17,262 | 63 | 260 | 0 |
| Griffon..... | 63 | 274 | 0 | 0 | 17,262 | 63 | 278 | 0 |
| Boxer | 53 | 246 | 0 | 0 | 13,038 | 53 | 97 | 0 |
| Hardy..... | 53 | 167 | 0 | 0 | 8,851 | — | — | — |

II.

| 1902. | | 1903. | | | | | Cases per 1000 complement. | | |
|---|---------------------|---------------|-----------------------|--|---|---------------------|----------------------------|-------|--------|
| No. of cases of Mediterranean Fever connected with Malta. | Men. Days in Malta. | Comple- ment. | No. of days in Malta. | No. of cases of Mediter- ranean Fever. | No. of cases of Mediter- ranean Fever connected with Malta. | Men. Days in Malta. | 1901. | 1902. | 1903. |
| 14 | 125,828 | 758 | 93 | 8 | 5 | 70,494 | 21·11 | 22·43 | 10·55 |
| 7 | 91,758 | 746 | 102 | 10 | 9 | 76,092 | 45·58 | 9·38 | 13·40 |
| 15 | 144,210 | 759 | 121 | 10 | 8 | 91,839 | 17·13 | 25·03 | 13·18 |
| 7 | 122,199 | 759 | 163 | 15 | 13 | 123,717 | 21·08 | 15·94 | 19·76 |
| 4 | 129,030 | 770 | 35 | 8 | 2 | 28,490 | 18·45 | 7·91 | 10·39 |
| 2 | 42,840 | — | — | — | — | — | 5·60 | 5·60 | — |
| 5 | 69,258 | — | — | — | — | — | 26·61 | 11·20 | — |
| — | — | — | — | — | — | — | 12·61 | — | — |
| 16 | 157,377 | 753 | 8 | 5 | 2 | 6,024 | 21·25 | 27·90 | 6·64 |
| 12 | 36,682 | — | — | — | — | — | 18·32 | 23·96 | 23·96 |
| 4 | 96,418 | — | — | — | — | — | 11·78 | 8·83 | — |
| 3 | 69,498 | 429 | 122 | 12 | 7 | 52,338 | 2·33 | 13·99 | 27·97 |
| 3 | 23,331 | 101 | 223 | 2 | 2 | 23,028 | 9·90 | 29·70 | 19·80 |
| 20 | 265,085 | 721 | 365 | 21 | 21 | 263,065 | 20·71 | 27·43 | 29·96 |
| 0 | 2,050 | — | — | — | — | — | 2·44 | 0·00 | — |
| 0 | 2,940 | — | — | — | — | — | 44·22 | 0·00 | — |
| 11 | 86,850 | 450 | 154 | 16 | 13 | 69,300 | 15·56 | 35·56 | 35·56 |
| 20 | 120,756 | — | — | — | — | — | 10·09 | 30·26 | — |
| 0 | 6,820 | 124 | 86 | 0 | 0 | 10,664 | 0·00 | 0·00 | 0·00 |
| 8 | 101,890 | 443 | 96 | 4 | 2 | 41,528 | 11·29 | 18·04 | 9·03 |
| 2 | 26,442 | 226 | 196 | 3 | 3 | 44,296 | 13·23 | 17·70 | 13·28 |
| 22 | 166,140 | 780 | 199 | 23 | 12 | 155,220 | 3·85 | 32·05 | 29·49 |
| 5 | 92,664 | 429 | 160 | 5 | 2 | 68,640 | 9·32 | 13·99 | 11·66 |
| — | — | 149 | 5 | 0 | 0 | 745 | 6·80 | — | 0·00 |
| 6 | 46,816 | 224 | 99 | 3 | 2 | 22,176 | 4·46 | 40·18 | 13·39 |
| 1 | 38,950 | — | — | — | — | — | 11·42 | 5·71 | — |
| 7 | 39,872 | 224 | 181 | 5 | 4 | 40,544 | 40·18 | 53·57 | 22·32 |
| 3 | 27,606 | 107 | 202 | 2 | 1 | 21,614 | 84·11 | 37·38 | 18·69 |
| — | — | — | — | — | — | — | 0·00 | — | — |
| 6 | 27,346 | 121 | 171 | 16 | 13 | 20,691 | 49·59 | 57·85 | 132·23 |
| 1 | 21,296 | 123 | 139 | 0 | 0 | 17,097 | 0·00 | 8·26 | 0·00 |
| — | — | — | — | — | — | — | 0·00 | — | — |
| 6 | 22,386 | 91 | 171 | 4 | 2 | 15,561 | 32·97 | 65·93 | 43·95 |
| 0 | 8,957 | — | — | — | — | — | 0·00 | 0·00 | — |
| 0 | 7,738 | — | — | — | — | — | 0·00 | 0·00 | — |
| 0 | 14,931 | 63 | 248 | 0 | 0 | 15,624 | 0·00 | 0·00 | 0·00 |
| 0 | 17,388 | 63 | 272 | 0 | 0 | 17,136 | 0·00 | 0·00 | 0·00 |
| 0 | 15,246 | 63 | 191 | 0 | 0 | 12,033 | 0·00 | 0·00 | 0·00 |
| 0 | 17,888 | 63 | 61 | 0 | 0 | 3,843 | 0·00 | 0·00 | 0·00 |
| 0 | 7,420 | 53 | 16 | 0 | 0 | 848 | 0·00 | 0·00 | 0·00 |
| 0 | 15,370 | 53 | 244 | 0 | 0 | 12,932 | 0·00 | 0·00 | 0·00 |
| 0 | 14,679 | — | — | — | — | — | 0·00 | 0·00 | — |
| 0 | 16,380 | 63 | 164 | 0 | 0 | 10,332 | 0·00 | 0·00 | 0·00 |
| 0 | 17,514 | 63 | 208 | 0 | 0 | 13,104 | 0·00 | 0·00 | 0·00 |
| 0 | 5,141 | 53 | 80 | 0 | 0 | 4,240 | 0·00 | 0·00 | 0·00 |
| — | — | — | — | — | — | — | 0·00 | — | — |

Table II

| Name of Ship. | 1901. | | | | | 1902. | | |
|-------------------|-------------|-----------------------|--------------------------------------|---|---------------------|-------------|-----------------------|--------------------------------------|
| | Complement. | No. of days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever connected with Malta. | Men. Days in Malta. | Complement. | No. of days in Malta. | No. of cases of Mediterranean Fever. |
| H.M.S.— | | | | | | | | |
| Orwell..... | 63 | 205 | 0 | 0 | 12,915 | 63 | 250 | 0 |
| Coquette..... | 63 | 253 | 0 | 0 | 15,939 | 63 | 242 | 0 |
| Cygnets..... | 63 | 186 | 0 | 0 | 11,718 | 63 | 242 | 0 |
| Conflict..... | 53 | 265 | 0 | 0 | 14,045 | — | — | — |
| Cruiser..... | 93 | 179 | 0 | 0 | 16,647 | 93 | 242 | 3 |
| Imogene..... | 42 | 75 | 0 | 0 | 3,150 | 53 | 91 | 0 |
| Formidable..... | 777 | 28 | 0 | 0 | 21,756 | 777 | 90 | 18 |
| Pandora..... | 226 | 10 | 0 | 0 | 2,260 | 226 | 174 | 0 |
| Irresistible..... | — | — | — | — | — | 780 | 145 | 5 |
| Repulse..... | — | — | — | — | — | 721 | 117 | 12 |
| Flying Fish..... | — | — | — | — | — | 63 | 175 | 0 |
| Goldfinch..... | — | — | — | — | — | 94 | 56 | 0 |
| Bulwark..... | — | — | — | — | — | 829 | 104 | 5 |
| Vengeance..... | — | — | — | — | — | 762 | 84 | 10 |
| Aboukir..... | — | — | — | — | — | 755 | 79 | 12 |
| London..... | — | — | — | — | — | 742 | 104 | 3 |
| Hermione..... | 318 | 12 | 0 | 0 | 3,816 | 324 | 141 | 11 |
| Ariel..... | 63 | 28 | 0 | 0 | 1,764 | 63 | 242 | 0 |
| Naiad..... | — | — | — | — | — | 276 | 76 | 3 |
| *Orion..... | — | — | — | — | — | 55 | 274 | 6 |
| Panther..... | — | — | — | — | — | 63 | 250 | 0 |
| Locust..... | — | — | — | — | — | 63 | 250 | 0 |
| Thrasher..... | — | — | — | — | — | 63 | 299 | 0 |
| St. George..... | 560 | 5 | 0 | 0 | 2,800 | 560 | 13 | 0 |
| Juno..... | 456 | 5 | 0 | 0 | 2,280 | 456 | 13 | 0 |
| Rainbow..... | — | — | — | — | — | 276 | 13 | 0 |
| Brilliant..... | — | — | — | — | — | 279 | 13 | 0 |
| Albatross..... | — | — | — | — | — | 69 | 137 | 0 |
| Fawn..... | — | — | — | — | — | 63 | 137 | 0 |
| Mallard..... | — | — | — | — | — | 63 | 123 | 0 |
| Cynthia..... | — | — | — | — | — | 63 | 126 | 0 |
| Stag..... | — | — | — | — | — | 63 | 14 | 0 |
| Bat..... | — | — | — | — | — | 63 | 14 | 0 |
| Seal..... | — | — | — | — | — | 63 | 14 | 0 |
| Crane..... | — | — | — | — | — | 63 | 14 | 0 |
| Venerable..... | — | — | — | — | — | 771 | 10 | 0 |
| Bacchante..... | — | — | — | — | — | 729 | 11 | 0 |
| Intrepid..... | — | — | — | — | — | 271 | 2 | 0 |
| Mohawk..... | 178 | 1 | 0 | 0 | 178 | — | — | — |
| Russell..... | — | — | — | — | — | — | — | — |
| Montagu..... | — | — | — | — | — | — | — | — |
| Exmouth..... | — | — | — | — | — | — | — | — |
| Albemarle..... | — | — | — | — | — | — | — | — |

* Since this table was made out, I have been informed that the cases returned as occurring on thus refer to a total complement of 1331 men. This accounts for the apparent absence of cases

—continued.

| 1902. | | 1903. | | | | | Cases per 1000 complement. | | |
|---|---------------------|-------------|-----------------------|--------------------------------------|---|---------------------|----------------------------|--------|--------|
| No. of cases of Mediterranean Fever connected with Malta. | Men. Days in Malta. | Complement. | No. of days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever connected with Malta. | Men. Days in Malta. | 1901. | 1902. | 1903. |
| 0 | 15,750 | 63 | 25 | 0 | 0 | 1,575 | 0·00 | 0·00 | 0·00 |
| 0 | 15,246 | 63 | 15 | 0 | 0 | 945 | 0·00 | 0·00 | 0·00 |
| 0 | 15,246 | 63 | 191 | 0 | 0 | 12,033 | 0·00 | 0·00 | 0·00 |
| — | — | — | — | — | — | — | 0·00 | — | — |
| 3 | 22,506 | 93 | 89 | 1 | 1 | 8,277 | 0·00 | 32·28 | 10·75 |
| 0 | 4,823 | 41 | 59 | 0 | 0 | 2,419 | 0·00 | 0·00 | 0·00 |
| 10 | 69,030 | 777 | 183 | 18 | 12 | 142,191 | 0·00 | 23·17 | 23·17 |
| 0 | 39,224 | 226 | 115 | 1 | 1 | 25,990 | 0·00 | 0·00 | 4·45 |
| 3 | 113,100 | 780 | 125 | 14 | 8 | 97,500 | — | 6·41 | 17·95 |
| 6 | 84,357 | 721 | 74 | 4 | 1 | 53,354 | — | 16·64 | 5·55 |
| 0 | 11,025 | 63 | 263 | 0 | 0 | 16,569 | — | 0·00 | 0·00 |
| 0 | 5,264 | — | — | — | — | — | — | 0·00 | — |
| 3 | 86,216 | 829 | 187 | 10 | 9 | 155,023 | — | 4·02 | 12·06 |
| 3 | 64,008 | 762 | 136 | 10 | 10 | 103,632 | — | 13·12 | 13·12 |
| 9 | 59,645 | 755 | 131 | 16 | 11 | 98,905 | — | 15·89 | 21·19 |
| 2 | 77,168 | 742 | 143 | 29 | 17 | 106,106 | — | 4·04 | 39·08 |
| 9 | 45,684 | 324 | 73 | 7 | 4 | 23,652 | 0·00 | 33·92 | 21·60 |
| 0 | 15,246 | 63 | 275 | 0 | 0 | 17,325 | 0·00 | 0·00 | 0·00 |
| 2 | 20,976 | 271 | 82 | 2 | 2 | 22,222 | — | 10·87 | 7·38 |
| 6 | 15,070 | 55 | 365 | 18 | 18 | 20,075 | — | 109·09 | 327·27 |
| 0 | 15,750 | 63 | 112 | 0 | 0 | 7,056 | — | 0·00 | 0·00 |
| 0 | 15,750 | 63 | 208 | 0 | 0 | 13,104 | — | 0·00 | 0·00 |
| 0 | 18,837 | 63 | 208 | 0 | 0 | 13,104 | — | 0·00 | 0·00 |
| 0 | 7,280 | — | — | — | — | — | 0·00 | 0·00 | — |
| 0 | 5,828 | — | — | — | — | — | 0·00 | 0·00 | — |
| 0 | 3,588 | — | — | — | — | — | — | 0·00 | — |
| 0 | 3,627 | — | — | — | — | — | — | 0·00 | — |
| 0 | 9,453 | 69 | 208 | 0 | 0 | 14,352 | — | 0·00 | 0·00 |
| 0 | 8,631 | 63 | 221 | 0 | 0 | 13,923 | — | 0·00 | 0·00 |
| 0 | 7,749 | 63 | 235 | 0 | 0 | 14,805 | — | 0·00 | 0·00 |
| 0 | 7,938 | 63 | 216 | 0 | 0 | 13,608 | — | 0·00 | 0·00 |
| 0 | 882 | 63 | 230 | 0 | 0 | 14,490 | — | 0·00 | 0·00 |
| 0 | 882 | 63 | 235 | 0 | 0 | 14,805 | — | 0·00 | 0·00 |
| 0 | 882 | 63 | 208 | 0 | 0 | 13,104 | — | 0·00 | 0·00 |
| 0 | 882 | 63 | 203 | 0 | 0 | 12,789 | — | 0·00 | 0·00 |
| 0 | 7,710 | 771 | 161 | 11 | 10 | 124,131 | — | 0·00 | 14·27 |
| 0 | 8,019 | 729 | 164 | 9 | 5 | 119,556 | — | 0·00 | 12·34 |
| 0 | 542 | 271 | 73 | 3 | 1 | 19,783 | — | 0·00 | 11·07 |
| — | — | 180 | 69 | 3 | 3 | 12,420 | 0·00 | — | 16·67 |
| — | — | 715 | 107 | 5 | 2 | 76,505 | — | — | 6·99 |
| — | — | 715 | 59 | 3 | 3 | 42,185 | — | — | 4·20 |
| — | — | 715 | 24 | 2 | 0 | 17,160 | — | — | 2·80 |
| — | — | 742 | 14 | 0 | 0 | 10,388 | — | — | 0·00 |

board H.M.S. "Orion" include cases which occurred on the torpedo-boat destroyer flotilla, and on board the torpedo boat destroyer flotilla.

Table II

| Name of Ship. | 1901. | | | | | 1902. | | |
|------------------------|------------------|--------------------------------|---|---|---------------------------|------------------|--------------------------------|---|
| | Comple- ment. | No. of Days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever connected with Malta. | Men. Days in Malta. | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. |
| H.M.S.— | | | | | | | | |
| Arethusa | — | — | — | — | — | — | — | — |
| Thetis | — | — | — | — | — | — | — | — |
| Hawke | — | — | — | — | — | 161 | 10 | 0 |
| Spartiate | — | — | — | — | — | — | — | — |
| Europa | — | — | — | — | — | — | — | — |
| Sirius | — | — | — | — | — | — | — | — |
| Victoria and Albert .. | — | — | — | — | — | — | — | — |
| Minerva | — | — | — | — | — | 456 | 6 | 0 |
| Venus | 452 | 17 | 0 | 0 | 7,684 | — | — | — |
| Assaye | — | — | — | — | — | — | — | — |
| Porpoise | 178 | 2 | 0 | 0 | 356 | — | — | — |
| Merlin | — | — | — | — | — | — | — | — |
| Pique | — | — | — | — | — | — | — | — |
| Cossack | — | — | — | — | — | — | — | — |
| Leviathan | — | — | — | — | — | — | — | — |
| Goliath | — | — | — | — | — | — | — | — |
| Seylla | — | — | — | — | — | — | — | — |
| Diadem | — | — | — | — | — | — | — | — |
| Psyche | — | — | — | — | — | — | — | — |
| Duncan | — | — | — | — | — | — | — | — |
| Centurion | 606 | 3 | 0 | 0 | 1,818 | — | — | — |
| Argonaut | — | — | — | — | — | — | — | — |
| Arrogant | — | — | — | — | — | — | — | — |
| Furious | — | — | — | — | — | 442 | 6 | 0 |
| Undaunted | 494 | 1 | 0 | 0 | 494 | — | — | — |
| Ringdove | 76 | 1 | 0 | 0 | 76 | — | — | — |
| Peacock | 76 | 2 | 0 | 0 | 152 | — | — | — |
| Magicienne | 224 | 1 | 0 | 0 | 224 | — | — | — |
| Raccoon | 182 | 1 | 0 | 0 | 182 | — | — | — |
| Linnet | 92 | 4 | 0 | 0 | 368 | — | — | — |
| Pigeon | 76 | 1 | 0 | 0 | 76 | — | — | — |
| Bonaventure | 318 | 1 | 0 | 0 | 318 | — | — | — |
| Marathon | 224 | 1 | 0 | 0 | 224 | — | — | — |
| Dido | 450 | 2 | 0 | 0 | 900 | — | — | — |
| Isis | 450 | 2 | 0 | 0 | 900 | — | — | — |
| Cockatrice | 78 | 1 | 0 | 0 | 78 | 78 | 1 | 0 |
| Melita | 125 | 2 | 0 | 0 | 250 | — | — | — |
| Ocean | 751 | 2 | 0 | 0 | 1,502 | — | — | — |
| Ophir | 324 | 2 | 0 | 0 | 648 | — | — | — |
| Rambler | 113 | 87 | 0 | 0 | 9,831 | — | — | — |
| Blake | 128 | 2 | 0 | 0 | 256 | — | — | — |
| Blenheim | 592 | 2 | 0 | 0 | 1,184 | — | — | — |
| Phœbe | 217 | 2 | 0 | 0 | 434 | — | — | — |
| Perseus | 224 | 1 | 0 | 0 | 224 | — | — | — |
| Talbot | 442 | 1 | 0 | 0 | 442 | — | — | — |
| Lapwing | 78 | 1 | 0 | 0 | 78 | — | — | — |

| Name of Ship. | 1901. | | | | | 1902. | | |
|---------------------|------------------|--------------------------------|---|--|---------------------------|------------------|--------------------------------|---|
| | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever con- nected with Malta. | Men. Days in Malta. | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. |
| H.M.S.— | | | | | | | | |
| Eclipse | 455 | 3 | 0 | 0 | 1,365 | — | — | — |
| Cressy | 754 | 1 | 0 | 0 | 754 | — | — | — |
| Albion | 791 | 4 | 0 | 0 | 3,164 | — | — | — |
| Fox | 324 | 3 | 0 | 0 | 972 | — | — | — |
| Iphigenia | 105 | 6 | 0 | 0 | 630 | 105 | 1 | 0 |
| Fearless | 149 | 1 | 0 | 0 | 149 | — | — | — |
| Mutine | 105 | 2 | 0 | 0 | 210 | — | — | — |
| Vestal | 105 | 1 | 0 | 0 | 105 | — | — | — |
| Amphitrite | 327 | 3 | 0 | 0 | 981 | 327 | 4 | 0 |
| Rinaldo | — | — | — | — | — | 105 | 2 | 0 |
| Espiègle | — | — | — | — | — | 113 | 3 | 0 |
| Daphne | — | — | — | — | — | 138 | 2 | 0 |
| Brisk | — | — | — | — | — | 180 | 10 | 0 |
| Aurora | — | — | — | — | — | 503 | 1 | 0 |
| Redpole | — | — | — | — | — | 78 | 1 | 0 |
| Plover | — | — | — | — | — | 78 | 3 | 0 |
| Pigmy | — | — | — | — | — | 78 | 1 | 0 |
| Astræa | — | — | — | — | — | 321 | 1 | 0 |
| Orlando | — | — | — | — | — | 503 | 2 | 0 |
| Endymion | — | — | — | — | — | 553 | 1 | 0 |
| Terrible | — | — | — | — | — | 870 | 2 | 0 |
| Majestic | — | — | — | — | — | 306 | 6 | 0 |
| Magnificent | — | — | — | — | — | 799 | 6 | 0 |
| Hannibal | — | — | — | — | — | 769 | 6 | 0 |
| Prince George | — | — | — | — | — | 766 | 6 | 0 |
| Jupiter | — | — | — | — | — | 769 | 6 | 0 |
| Mars | — | — | — | — | — | 766 | 6 | 0 |
| Niobe | — | — | — | — | — | 689 | 6 | 0 |
| Sutlej | — | — | — | — | — | 755 | 6 | 0 |
| Doris | — | — | — | — | — | 426 | 6 | 0 |
| Pactolus | — | — | — | — | — | 229 | 6 | 0 |
| Prometheus | — | — | — | — | — | 229 | 6 | 0 |

Summary of Table II.

| Totals. | 1901. | 1902. | 1903. |
|--|-----------|-----------|-----------|
| Number of cases of Mediterranean Fever .. | 249 | 349 | 338 |
| Number of cases of Mediterranean Fever connected with Malta | 198 | 266 | 241 |
| Men—days in Malta | 2,810,819 | 3,319,849 | 2,882,323 |
| Average men constantly in Malta | 7,700·87 | 9,095·48 | 7,896·77 |
| Cases per 1000 complement | 25·71 | 29·25 | 30·51 |
| 3 years' average | 28·55 | | |

continued.

| 1902. | | 1903. | | | | | Cases per 1000 complement. | | |
|---|---------------------------|------------------|--------------------------------|---|--|---------------------------|----------------------------|-------|-------|
| No. of ases of fediterranean Fever con- nected with Malta. | Men. Days in Malta. | Comple- ment. | No. of days in Malta. | No. of cases of Mediterranean Fever. | No. of cases of Mediterranean Fever con- nected with Malta. | Men. Days in Malta. | 1901. | 1902. | 1903. |
| — | — | — | — | — | — | — | 0·00 | | |
| — | — | — | — | — | — | — | 0·00 | | |
| — | — | — | — | — | — | — | 0·00 | | |
| — | — | — | — | — | — | — | 0·00 | | |
| 0 | 105 | — | — | — | — | — | 0·00 | 0·00 | |
| — | — | — | — | — | — | — | 0·00 | | |
| — | — | — | — | — | — | — | 0·00 | | |
| 0 | 1,308 | — | — | — | — | — | 0·00 | 0·00 | |
| 0 | 210 | — | — | — | — | — | — | 0·00 | |
| 0 | 339 | — | — | — | — | — | — | 0·00 | |
| 0 | 276 | — | — | — | — | — | — | 0·00 | |
| 0 | 1,800 | — | — | — | — | — | — | 0·00 | |
| 0 | 503 | — | — | — | — | — | — | 0·00 | |
| 0 | 78 | — | — | — | — | — | — | 0·00 | |
| 0 | 234 | — | — | — | — | — | — | 0·00 | |
| 0 | 78 | — | — | — | — | — | — | 0·00 | |
| 0 | 321 | — | — | — | — | — | — | 0·00 | |
| 0 | 1,006 | — | — | — | — | — | — | 0·00 | |
| 0 | 553 | — | — | — | — | — | — | 0·00 | |
| 0 | 1,740 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,836 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,794 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,614 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,596 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,614 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,596 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,184 | — | — | — | — | — | — | 0·00 | |
| 0 | 4,530 | — | — | — | — | — | — | 0·00 | |
| 0 | 2,556 | — | — | — | — | — | — | 0·00 | |
| 0 | 1,374 | — | — | — | — | — | — | 0·00 | |
| 0 | 1,374 | — | — | — | — | — | — | 0·00 | |

often absent from Malta during the season when Mediterranean Fever is at its worst.

It appears that when Mediterranean Fever attacks the crew of a ship one year, it is very likely to attack the crew of the same ship in each of the following years that she remains on the station.* There is little relation between the number of days spent by a ship in Malta and the number of cases of Mediterranean Fever which occur

* H.M.S. "Rupert" left the Mediterranean in February, 1902.

on board her, but since no allowance is made in the table for the time of year at which the ship was in Malta, the relation may be closer or less close than is apparent here.

The facts seem to point to the ship herself becoming infected, and in some way assisting in the transmission of infection. Of course, the larger the crew the greater the chance of infection being introduced into the ship from outside. It must be remembered, however, that I am only dealing with a three-year period, which is altogether too short to base reliable conclusions upon.

The data from which the above table is compiled did not reach me until some time after I left Malta, so that I was unable to make investigation by its light on board ships which were at Malta during my visit. It may be said, however, that on larger ships the ventilation is better, and that on Destroyers, in particular, the closets are so constructed that they must be very difficult to cleanse.

The distribution of Mediterranean Fever amongst the garrison during the period 1897 to 1903 is shown in the following table, which

Table III.

| | Number of cases of Mediterranean Fever admitted to hospital during the period 1897—1903. | Average number of men in occupation during each year of the period 1897—1903. | Average number of cases admitted per 1000 of strength per year during the period 1897—1903. |
|--|---|--|--|
| St. Francis, Floriana (R.E.) and Ravelin | 20 | 194·86 | 14·7 |
| Floriana (infantry) | 249 | 572·14 | 62·2 |
| Lower St. Elmo (infantry) | 134 | 649·57 | 29·4 |
| Upper " (R.G.A.) | 46 | 272·86 | 24·0 |
| Tigne (R.G.A.) | 54 | 412·57 | 18·7 |
| Manoel (infantry), including huts ... | 133 | 832·86 | 22·8 |
| Notre Dame (infantry), including Ravelin and huts | 49 | 217·14 | 32·2 |
| Old laboratory (infantry and A.S.C.) .. | 17 | 86·43 | 28·1 |
| Marsamuscetto (infantry and A.O.C.) .. | 18 | 74·43 | 34·5 |
| St. James' Cavalier (R.G.A.) | 12 | 129·86 | 13·2 |
| Verdala (infantry) | 104 | 534·14 | 27·8 |
| Imtarfa (infantry and various) | 224 | 989·29 | 13·7 |
| Porte de Bombe tents | 1 | No record | available. |
| Castille (R.A. and R.E.) | 1 | 17·86 | 8·0 |
| Fort Madelina (R.G.A.) .. | 1 | 25·71 | 5·6 |
| Valetta Hospital (R.A.M.C.) | 29 | 65·00 | 65·2 |
| Mellieha Camp | 33 | 273·40* | 24·1* |
| Corradino Prison | 6 | 16·00 | 53·6 |

* For period 1899—1903, not occupied before 1899.

Table III—continued.

| | Number of cases of Mediterranean Fever admitted to hospital during the period 1897—1903. | Average number of men in occupation during each year of the period 1897—1903. | Average number of cases admitted per 1000 of strength per year during the period 1897—1903. |
|---|---|--|--|
| Camarata married quarters | 15 | 82·36 | 25·9 |
| Sliema married quarters | 7 | No record | available. |
| St. Clements (infantry) | 5 | 50·00 | 14·3 |
| Zabbar Gate „ | 10 | 93·86 | 15·2 |
| Zeitun Gate „ | 4 | 55·43 | 10·3 |
| Polverista „ | 35 | 156·86 | 31·9 |
| St. Nicholas „ | 4 | 33·71 | 16·9 |
| Vittoriosa „ | 3 | 47·43 | 9·0 |
| Salvatore „ (including C. Guard) | 2 | 96·43 | 2·1 |
| Fort St. Angelo (R.M.A. and infantry) | 2 | 83·71 | 3·4 |
| Couvre Porte (infantry and R.M.A.) | 1 | 51·43 | 2·8 |
| Inquisitor's Palace (infantry) | 3 | 7·29 | 58·8 |
| St. Paul's Bastion (infantry and A.S.C. stables) | 4 | 26·29 | 21·7 |
| St. John's Bastion (infantry) | 1 | 22·43 | 6·4 |
| Fort Ricasoli (R.G.A.) | 40 | 495·14 | 11·5 |
| „ Rinella „ | 1 | 15·86 | 9·0 |
| „ Delimara „ | 6 | 23·43 | 36·6 |
| „ San Leonardo (R.A. and R.E.) | 3 | 25·14 | 17·0 |
| „ della Grazia (R.G.A.) | 2 | 13·00 | 22·0 |
| „ San Francesco (infantry) | 2 | 23·71 | 12·0 |
| „ Isola Gate (infantry) | 8 | 39·14 | 29·2 |
| Cottonera Hospital (R.A.M.C.) | 25 | 44·14 | 80·9 |
| Pembroke (R.G.A. and infantry) | 49 | 187·14 | 37·4 |
| Duerra Lines (R.G.A.) | 1 | 4·57 | 31·9 |
| Ghain Tuffieha | 99 | 370·67* | 89·0* |
| St. George's Barracks (R.G.A. and infantry) | 108 | 1024·71 | 15·1 |
| Forrest Hospital (R.A.M.C.) | 1 | 9·43 | 15·1 |
| Gozo | 24 | 199·86 | 17·2 |
| Fort Lascaris (R.M.A.) | 29 | 253·86 | 16·3 |
| Living in places not mentioned above | 0 | 443·29 | 00·0 |
| Whole garrison | 1625 | 9037·43 | 25·6 |

is compiled from information furnished me by the Director of Transport and Supplies, and by the Principal Medical Officer in Malta. The cases are only those admitted to hospital, and would include all cases occurring amongst the garrison except a few officers who were nursed at their own homes. Of these I could not obtain a record.

The average annual attack rate for the whole garrison, as far as

* For years 1901, 1902, and 1903, not occupied before 1901.

TABLE IV.

| Age | 1902. | | | | | 1903. | | | | 1902 and 1903. |
|------------------------|-------------------|--|-----------------------------|--------------------------------------|---------|-------------------|--|-----------------------------|--------------------------------------|--------------------------------------|
| | Average strength. | Admissions to hospital on account of Malarian Fever. | Deaths from Malarian Fever. | Ratio per 1000 of strength per year. | | Average strength. | Admissions to hospital on account of Malarian Fever. | Deaths from Malarian Fever. | Ratio per 1000 of strength per year. | Ratio per 1000 of strength per year. |
| | | | | | | | | | | |
| | | | | Admissions. | Deaths. | | | | Admissions. | Deaths. |
| Under 20 years | 1082 | 14 | 0 | 13.6 | 0.00 | 1041 | 67 | 3 | 64.4 | 2.88 |
| From 20 to 25 years .. | 2939 | 45 | 1 | 15.3 | 0.34 | 3460 | 196 | 1 | 56.6 | 0.29 |
| " 25 30 " .. | 1926 | 18 | 1 | 9.3 | 0.52 | 1495 | 53 | 1 | 35.4 | 0.67 |
| " 30 35 " .. | 1498 | 24 | 3 | 16.0 | 2.00 | 1117 | 34 | 2 | 30.4 | 1.79 |
| " 35 40 " .. | 1598 | 39 | 0 | 24.5 | 0.00 | 1294 | 37 | 1 | 28.5 | 0.77 |
| 40 years and upwards. | 280 | 15 | 1 | 65.2 | 4.35 | 386 | 17 | 1 | 44.0 | 2.59 |
| Totals | 9218 | 155 | 6 | 16.8 | 0.65 | 8793 | 404 | 9 | 45.9 | 1.02 |
| | | | | | | | | | | 31.0 |

this record goes, was 25·6 per 1000 during the seven-year period 1897 to 1903. The heaviest incidence was upon Floriana Infantry Barracks, Valetta Station Hospital, Cottonera Hospital, and Ghain Tuffieha Camp. The two* first places are specially dealt with subsequently; the heavy incidence upon Ghain Tuffieha Camp was occasioned by an outbreak in 1903. A large number of men annually occupy the camp for a few months during the summer only, so that an outbreak occurring amongst them raises the figure representing the annual case incidence out of proportion to the importance of the outbreak itself, because the representative figure is calculated on the average occupation during the whole year.

Age and Sex Incidence could not be ascertained from the civil official returns, but next autumn figures which are now being collected for the year ending July 31, 1905, will be available.

The age incidence for the garrison during 1902 and 1903 is shown as far as possible in Table IV (see page 32).

The two years tabulated show considerable disparity in the number of admissions and in the resulting ratios per 1000 of strength. From the figures of the two years together, it would appear that men under 25 years of age suffer more than the average number of attacks per 1000. From 25 to 40 there is a degree of immunity becoming less with advancing years. Over age 40 the incidence again becomes severe. The deaths are too few to permit of any useful deductions being made from them.

Length of Service in Malta is, as might be expected, closely connected with age in its influence upon the incidence of Mediterranean Fever, as the following table shows (see page 34).

The heaviest incidence of Mediterranean Fever is upon men with less than one year's service; in fact, they are the only class shown with an incidence greater than the average incidence of the disease upon all classes taken together. The incidence upon men with over two years' service is less than half that upon men with under two years' service, and the severity of incidence continues to decrease with length of service up to five years, after which it again rises. In the last three classes shown in the table, however, the numbers dealt with are too small to carry much weight.

The decrease of incidence with length of service in Malta is, no doubt, influenced to a large extent by the elimination of the more susceptible subjects. Further figures will be available next autumn.

The Case Mortality amongst the civil population differs enormously in different localities, as is to be expected when dealing with small numbers and unreliable notification returns. Amongst the civil population of Malta, 8·9 cases per 100 attacked died during the period

* There was an outbreak of Mediterranean Fever in Floriana infantry barracks during 1903. The details were not obtained in time for inclusion in this report.

TABLE V.

| Length of service in Malta. | 1902. | | | | | 1903. | | | | | 1902 and 1903. | |
|--------------------------------|----------------------|--|---|--|---------|----------------------|--|---|--|---------|---|--|
| | Average strength. | Admissions to hospital on account of Mediterranean Fever. | Deaths from Mediterranean Fever. | Ratio per 1000 of strength per year. | | Average strength. | Admissions to hospital on account of Mediterranean Fever. | Deaths from Mediterranean Fever. | Ratio per 1000 of strength per year. | | Ratio per 1000 of strength per year. | |
| | | | | Admissions. | Deaths. | | | | Admissions. | Deaths. | | |
| | | | | | | | | | | | | |
| Under 1 year..... | 4878 | 103 | 3 | 21.1 | 0.62 | 4543 | 261 | 2 | 57.5 | 0.44 | 38.6 | |
| From 1 to 2 years..... | 1913 | 30 | 1 | 15.7 | 0.52 | 3142 | 117 | 5 | 37.2 | 1.59 | 29.1 | |
| " 2 3 "..... | 1190 | 10 | 0 | 8.4 | 0.00 | 550 | 16 | 2 | 29.1 | 3.64 | 14.9 | |
| " 3 4 "..... | 550 | 6 | 1 | 10.9 | 1.82 | 259 | 6 | 0 | 23.2 | 0.00 | 14.8 | |
| " 4 5 "..... | 309 | 3 | 0 | 9.7 | 0.00 | 173 | 0 | 0 | 00.0 | 0.00 | 6.2 | |
| " 5 10 "..... | 292 | 2 | 1 | 6.8 | 3.42 | 106 | 4 | 0 | 37.7 | 0.00 | 15.1 | |
| 10 years and upwards. | 86 | 1 | 0 | 11.6 | 0.00 | 20 | 0 | 0 | 00.0 | 0.00 | 9.4 | |
| Totals | 9218 | 155 | 6 | 16.8 | 0.65 | 8793 | 404 | 9 | 45.9 | 1.02 | 31.0 | |

1894 to 1903, and amongst the civil population in Gozo, 8.4 per 100 attacked died during the same period. These figures form a striking contrast to the case mortality of the Army and of the Navy. In the Army, during the period 1897 to 1903, the case mortality was 3.2 per cent., and in the Navy it was only 1.4 per cent. during the period 1897 to 1901. It is probable that the case mortality is higher amongst the civil population than in the Army and Navy, owing to the superior nursing and attention enjoyed by the services, but I do not think it likely that the difference noted above represents the true state of affairs. Probably the high case mortality among the civilians is largely due to the fact that mild cases of Mediterranean Fever more often escape notification than severe ones.

Temperature and Rainfall in Connection with Mediterranean Fever.—No official data were available with regard to temperature and rainfall for the whole of the period 1894 to 1903. The curves in the accompanying chart are constructed from figures kindly supplied me by the Rev. Father J. F. Dobson, S.J., the result of observations made at St. Julian's, near Valetta. I have inserted also a curve representing the case incidence of Mediterranean Fever, for comparison. The last-named curve is based on figures taken from the civil official notification records (see next page).

It will be at once seen that there is a very close correspondence between the curve representing the temperature and that representing the number of cases. The rise of the latter curve follows that of the former at an interval of about one month, which would be approximately sufficient to allow for incubation and notification if the incidence of fever were directly dependent upon the temperature of the air. The temperature curve attains its maximum in July and continues high during August, after which it begins to drop. The case curve attains its maximum a month later, but, unlike the temperature curve, it at once commences to drop, so that it would appear that whatever connection the air temperature may have with case incidence, does not remain so obvious after the former has attained its maximum.

The curve representing rainfall is in general the inverse of that representing temperature. It attains its minimum in July, but it is almost as low in June and August. The "case" curve commences to drop at the same time that the rainfall curve commences to rise, allowing no interval for incubation and notification, so that the connection is not clear; nor does the steep rise of the rainfall curve, at the end of September, produce a correspondingly steep decline in the case curve as might have been expected were the connection between the two intimate.

Seasonal Prevalence of Mediterranean Fever.—On p. 37 will be found a table giving the number of cases that were notified each month of

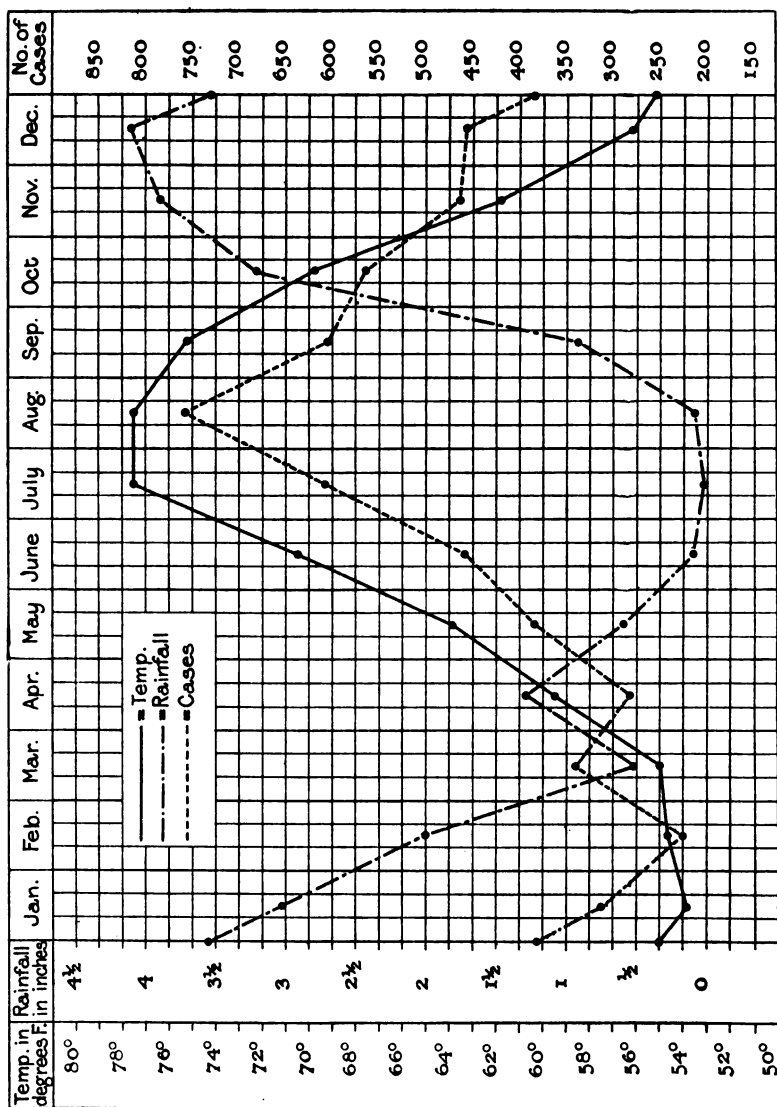


Chart showing temperature, rainfall, and number of cases amongst the civil population during period 1894—1908.

each year of the period 1894 to 1903, together with the total number of cases which were notified during the period in each month.

The figures in Table VI probably represent with some accuracy the seasonal prevalence of Mediterranean Fever, because though they are founded upon the civil official notification returns, there does not appear to be any particular reason why these returns should be more inaccurate at one time of the year than at another.

Table VI.

| | Jan. | Feb. | Mar. | Apr. | May. | June. | July. | Aug. | Sept. | Oct. | Nov. | Dec. |
|-------|------|------|------|------|------|-------|-------|------|-------|------|------|------|
| 1894 | 22 | 6 | 35 | 28 | 24 | 18 | 44 | 22 | 53 | 30 | 16 | 21 |
| 1895 | 10 | 9 | 21 | 23 | 11 | 26 | 16 | 47 | 42 | 18 | 27 | 12 |
| 1896 | 18 | 11 | 16 | 27 | 70 | 69 | 126 | 145 | 71 | 33 | 31 | 30 |
| 1897 | 28 | 25 | 22 | 30 | 52 | 50 | 92 | 89 | 49 | 47 | 41 | 38 |
| 1898 | 41 | 16 | 17 | 20 | 36 | 32 | 49 | 40 | 50 | 64 | 54 | 88 |
| 1899 | 61 | 38 | 49 | 39 | 47 | 63 | 105 | 77 | 58 | 103 | 91 | 71 |
| 1900 | 38 | 30 | 37 | 33 | 37 | 42 | 65 | 83 | 79 | 78 | 82 | 44 |
| 1901 | 46 | 32 | 25 | 24 | 41 | 67 | 74 | 89 | 78 | 64 | 43 | 42 |
| 1902 | 17 | 34 | 37 | 34 | 33 | 37 | 66 | 84 | 71 | 61 | 49 | 65 |
| 1903 | 32 | 26 | 31 | 23 | 35 | 54 | 68 | 82 | 54 | 71 | 33 | 44 |
| Total | 313 | 222 | 290 | 281 | 386 | 458 | 605 | 758 | 605 | 569 | 467 | 455 |

The maximum prevalence would appear to be in the month of August and the minimum in February. It is to be noted that the number of cases notified during February does not approach zero, being roughly 30 per cent. of the number notified in August.

From February to August there is each month a steady increase in the number of cases, except for a slight drop-back in April, and from August to February a steady decrease.

Consideration of Ways in which the Infection of Mediterranean Fever may be Transmitted.

(1) *Direct Personal Infection* (that is, by contact, or by the breath, or by the saliva).—No reliable data as to the number of dwellings in which more than one case of Mediterranean Fever occurred, could be obtained from the official notification records. The name of the patient was seldom given, and on visiting an address which had appeared more than once in the records, it was generally found to be a tenement house occupied by 15 or 20 families. Often there was no record of the number of the house, the street only being indicated.

Amongst 100 houses which I personally visited, in all of which Mediterranean Fever had been notified during 1904, I only found six houses in which there had been more than one case notified. There was often strong probability that other cases of a lighter nature than that notified had occurred, but the information elicited was never conclusive.

In Malta, outside Valetta, Cospicua, Vittoriosa, and Senglea, as noted above, both density of population upon area, and aggregation of a large number of persons in one locality appear to favour the spread of Mediterranean Fever. This may be because such conditions give greater opportunity for close personal contact, and so for direct personal infection. In making any inference it must not be forgotten

that Valetta and the three cities are exceptions, although both conditions are present in an eminent degree. It would appear, therefore, that whatever may be the conditions which favourably differentiate Valetta and the three cities from the rest of Malta, they must have an enhanced importance in their bearing upon the spread of Mediterranean Fever, inasmuch as they seem to more than counterbalance two conditions which appear to favour the spread of the disease in other parts of Malta.

Thirty-five women, wives of non-commissioned officers in the garrison, were attacked by Mediterranean Fever during 1904. Only two of them were removed to hospital. If direct personal infection were always an important factor in the spread of Mediterranean Fever, it would be expected that a large proportion of the husbands of these women would have been attacked, yet only five fell ill. Moreover, in two cases out of the five the husband and wife appear to have fallen ill on the same day, which would point rather to infection from a common source, than to infection from husband to wife, or *vice versa*.

Set out in the following table is a comparison between the three principal hospitals of Malta, showing separately the incidence of Mediterranean Fever upon the nursing staffs, and upon the patients undergoing treatment in hospital for other diseases, during the five years 1899 to 1903 respectively. I have added to the table, for comparison, the incidence of Mediterranean Fever upon the troops quartered in Valetta during the same period.

The number of patients per 1000 constantly ill, in other words, per mean yearly number of patients in hospital, is not strictly comparable with that of the staff per 1000 of strength, because in the former we are dealing with a shifting population in which fresh patients would constantly become exposed to infection, if the hospital were a centre of infection, whereas the staff would not vary in the same degree. In addition, the patients in hospital would be more numerous in summer, owing to "simple continued fever," than in winter.

It will be seen that during the period the average incidence of Mediterranean Fever upon patients treated in the Station Hospital, Valetta, was less than that upon the troops quartered in Valetta, while in the other two hospitals it was much greater. In all three hospitals very severe incidence occurred on the nursing staffs.

For the better consideration of the difference of incidence upon the respective staffs of the three hospitals I add here a short note upon each.

The Station Hospital, Valetta, has only attempted to isolate Mediterranean Fever since 1903. The isolation is incomplete; the Mediterranean Fever wards are separated from other wards only by a wall reaching about half-way to the ceiling. Enteric cases are frequently put in the Mediterranean Fever wards, and I have seen at least one case of Mediterranean Fever in a general ward.

Table VII.

| | Valetta Station Hospital. | | | Central Civil Hospital. | | | R.N. Hospital, Bighi. | | | Valetta troops. |
|--------|--|---|--|--|---|--|--|---|--|-----------------|
| | No. of cases of Mediteranean Fever admitted. | No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.* | No. of cases of Mediteranean Fever which occurred amongst hospital orderlies per 1000 of strength. | No. of cases of Mediteranean Fever admitted. | No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.* | No. of cases of Mediteranean Fever which occurred amongst hospital nursing staff per 1000 of strength. | No. of cases of Mediteranean Fever admitted. | No. of cases of Mediteranean Fever which occurred amongst hospital patients per 1000 constantly ill.* | No. of cases of Mediteranean Fever which occurred amongst sick bay staff per 1000 of strength. | |
| 1899.. | 84 | 32·00 | 34·48 | 129 | 58·44 | 171·4 | 111 | 66·67 | 83·3 | 22·54 |
| 1900.. | 85 | 6·44 | 54·03 | 147 | 100·63 | 171·4 | 261 | 89·55 | 459·5 | 26·18 |
| 1901.. | 127 | 45·75 | 121·21 | 127 | 96·15 | 138·9 | 175 | 78·43 | 122·0 | 43·11 |
| 1902.. | 60 | 34·18 | 48·78 | 127 | 47·62 | 111·1 | 272 | 175·50 | 476·2 | 16·90 |
| 1903.. | 222 | 12·26 | 50·00 | 154 | 83·80 | 83·2 | 279 | 102·74 | 150·3 | 17·81 |
| | | 25·9 | 56·5 | — | 77·2 | 134·8 | — | 105·18 | 289·34 | 34·31 |

The number of cases which occurred amongst hospital patients was calculated for each hospital by reckoning all cases of Mediterranean Fever which occurred in patients more than 14 days after their admission, and less than 14 days after their discharge. Patients who were admitted suffering from a disease that might have been Mediterranean Fever are excluded.

The means provided for the disposal of bed-pan contents and slops are wholly inadequate. A separate slop sink is used for enteric and Mediterranean Fever cases only. It is, however, on a different landing to the wards, and at a considerable distance. It is not supplied with a proper flush for cleansing purposes, and it is in a small dark room with faulty pavement. Some bed-pans examined by me were found to be very foul, though supposed to be cleansed. Izal is used for disinfecting the bed-pans, and carbolic solution for the orderlies' hands. The patients are cleansed with carbolic solution after the bed-pan has been used, and izal is put in the bed-pan before use.

Water-closets are used by enteric and Mediterranean Fever convalescents in common with other convalescents. The urinal provided is of the perpendicular slab type with an insufficient flush. It smelt offensively and the floor was soiled with urine.

The hospital orderlies spend about 50 hours per week in the wards.

The Royal Naval Hospital, Bighi, has isolated Mediterranean Fever for the past three years in special wards. These wards are amply provided with modern hospital sinks containing powerful flushes. Three of the sinks in the hospital were not ready for immediate use at the time of my visit. Izal is placed in the bed-pan before use, and an india-rubber sheet is put under the patient. The sheet shown me was perished and soiled. The patient is cleaned with soap and water, but the attendant does not disinfect his hands as a routine matter.

The sick-bay staff spend between 66 and 71 hours in the wards per week. Soiled linen is conveyed to the laundry by the sick-bay staff and washed with the other linen.

The Central Civil Hospital.—No attempt is made here to isolate patients suffering from Mediterranean Fever. They are distributed at haphazard throughout the medical wards.

Bed-pans are emptied into a gully in an open space between the wards. The edges of the gully are protected by a metal funnel, but the bed-pans are carried carelessly across the open space, dripping portions of their contents on the pavement, and are roughly washed out at a hot-water tap over an ordinary grating.

The attendant does not cleanse his hands after the operation, and the bed-pan is not usually disinfected, though occasionally a small portion of "carbolic" powder is dusted into it. No attempt was made to cleanse the patient at the time of my visit, and his person and bed proved upon inspection to be in a filthy condition.

Infected clothing is said to be steeped in tubs containing a 1 in 1000 solution of corrosive sublimate before removal to the laundry at the poor-house. At the time of my visit there was no infected clothing in the two small tubs shown me, and I was informed that the liquid they contained was not corrosive sublimate solution.

The nurses spend practically all their time in the wards, eating

and sleeping there; but they have a holiday every third day from midday until 6 the next morning, or if it happen to be a visiting day (Wednesday or Sunday), they leave at 4 P.M. instead of midday. In addition, they have a holiday every three weeks from 9 A.M. to 6 A.M. the following day.

Male ward cleaners have the same hours except that they leave hospital on alternate days at 5 P.M., returning at 6 A.M., and on alternate Sundays from 8 A.M. to 6 A.M. on Monday.

Briefly, the Military Station Hospital has practised partial isolation during 1903.

The Royal Naval Hospital has practised complete isolation during the past three years.

The Central Civil Hospital has made no attempt at isolation.

The Military Hospital and the Naval Hospital take precautions against the spread of infection by excreta; while in the Civil Hospital such precautions are almost altogether neglected.

The incidence upon the staffs of the three hospitals is not in proportion to the precautions taken against infection, nor to the number of hours spent in the wards.

The patients and attendants in the civil hospital are entirely Maltese, and if the incidence upon either the one or the other be compared with the incidence upon the civil population of Malta as a whole, the result is remarkable. The attendants show an incidence of 134·8 per 1000, the patients 77·2 per 1000 constantly ill, while the civil population of Malta shows only 3·2 per 1000 during the 10 years, 1894 to 1903. These results are no doubt due largely to faulty notification.

It is possible that so-called endemic acquired immunity may play a part in reducing the incidence on the patients and attendants in the civil hospital, and that such immunity may invalidate comparisons with hospitals occupied altogether by Englishmen.

If the Valetta Station Hospital be compared with the Naval Hospital it will be found that the incidence of Mediterranean Fever upon the respective staffs and patients is not in proportion to the amount of isolation attempted, but is more or less in proportion to the amount of care exercised in disposing of the excreta of patients, and the number of hours spent in the wards by the attendants.

The cause of special incidence of Mediterranean Fever in the hospitals does not, on the evidence obtained, appear to be direct personal infection, since that would probably be more evident in the hospital where least isolation is attempted. The incidence, mentioned above, on non-commissioned officers whose wives suffered from Mediterranean Fever points in the same direction. Neither is the evidence in favour of a place infection, to which the patients would probably be more exposed

than the staff, and would, in addition, constantly present fresh material.

It is possible that an aggregation of cases of Mediterranean Fever in one place may be more infective than the same number spread over a large area, but we have no evidence to point to this. In the Naval Hospital during 1904—a very short period, no doubt—only one case of Mediterranean Fever arose amongst the patients occupying the adjoining wards to the Mediterranean Fever wards. A table showing the cases of Mediterranean Fever which occurred amongst patients in the Naval Hospital during 1904 will be found on p. 49.

(2) *Excretal infection* may occur by means of infected dust inhaled or swallowed, by contamination of the hands, and thence the mouth, or by contamination of food or drink.

Dust is very prevalent out of doors in Malta, because of the friable nature of the rock and the hot sun, and owing to the high winds prevalent, opportunity of inhaling or swallowing it is present during the greater part of the year. There is, without doubt, possibility of frequent pollution of the dust by the excreta of mild or unrecognised cases, more especially in the suburban area, where conveniences are few and scavenging desultory. Horrocks has shown that the *Micrococcus melitensis* is found in the urine of Mediterranean Fever patients, and that it can live for long periods in a desiccated condition.

The seasonal curves of temperature and rainfall are such that the degree of dust prevalence corresponds closely with the degree of Mediterranean Fever prevalence. It is true that the Mediterranean Fever curve begins to fall immediately after attaining its maximum, while the curve of temperature remains high and the curve of rainfall low; but that may be accounted for by the fact that the "Sciroc" wind begins to blow in August. I am informed that this wind is so laden with moisture that it renders the roads damp during its prevalence in August and September. I cannot say I observed this phenomenon during my stay in Malta, but during the summer of 1904 there was very little "Sciroc," although the temperature was unusually high.

The rainfall curve is on the whole consistent with the theory of dust infection, being in general the inverse of the Mediterranean Fever curve; but I understand that during long periods in the rainy season there is little or no dust. If this be so, and dust be largely concerned in the spread of infection, it would be expected that there would be corresponding periods almost free from Mediterranean Fever notifications, which is not the case.

I have made out the following table with a view to comparing the incidence during the most dusty months of the period 1894 to 1903 with that during the least dusty months, for the three areas, urban, suburban, and rural.

| | Jan. | Feb. | March. | April. | May. | June. | July. | Aug. | Sept. | Oct. | Nov. | Dec. |
|-------------------------------|------|------|--------|--------|------|-------|-------|------|-------|------|------|------|
| Urban area— | | | | | | | | | | | | |
| Valetta | 19 | 14 | 30 | 12 | 20 | 23 | 63 | 40 | 37 | 20 | 33 | 29 |
| Floriana | 15 | 5 | 16 | 16 | 14 | 36 | 33 | 40 | 29 | 26 | 23 | 17 |
| Cospicua | 8 | 8 | 4 | 7 | 8 | 45 | 38 | 28 | 16 | 21 | 10 | 8 |
| Vittoriosa | 8 | 7 | 6 | 3 | 7 | 20 | 15 | 23 | 28 | 21 | 12 | 14 |
| Senglea | 5 | 1 | 3 | 3 | 7 | 9 | 8 | 11 | 17 | 7 | 5 | 8 |
| Suburban area— | | | | | | | | | | | | |
| Misda and Pietà | 55 | 35 | 59 | 41 | 56 | 133 | 157 | 142 | 127 | 95 | 83 | 76 |
| Sliema and St. Julian's | 14 | 10 | 6 | 6 | 7 | 16 | 35 | 41 | 24 | 20 | 15 | 14 |
| Hamrun | 25 | 15 | 22 | 26 | 28 | 36 | 75 | 58 | 63 | 53 | 21 | 48 |
| Birchirchara | 30 | 26 | 35 | 38 | 43 | 46 | 63 | 117 | 63 | 69 | 44 | 33 |
| Birchirchara | 37 | 29 | 26 | 27 | 25 | 34 | 66 | 45 | 48 | 45 | 32 | 27 |
| Curmi | 1 | 4 | 3 | 2 | 2 | 3 | 6 | 4 | 3 | 5 | 6 | 1 |
| Tarxien and Paola | 5 | 6 | 5 | 7 | 11 | 10 | 9 | 10 | 6 | 10 | 13 | 9 |
| Zabbar | 19 | 10 | 23 | 7 | 18 | 14 | 22 | 25 | 19 | 22 | 18 | 17 |
| Rural area— | | | | | | | | | | | | |
| Notabile and Rabato | 131 | 100 | 120 | 113 | 134 | 159 | 276 | 308 | 226 | 224 | 149 | 149 |
| Dingli | 15 | 10 | 7 | 18 | 14 | 18 | 40 | 24 | 21 | 33 | 13 | 30 |
| Zebbug | 1 | 2 | — | 2 | 1 | 2 | 2 | 6 | 8 | 5 | — | 1 |
| Siggei | 16 | 12 | 15 | 16 | 25 | 12 | 28 | 46 | 37 | 31 | 30 | 22 |
| Lie, Attard, and Balzan | 3 | 3 | 6 | 8 | 5 | 5 | 14 | 12 | 11 | 8 | 5 | 11 |
| Naxaro | 10 | 3 | 12 | 28 | 37 | 19 | 49 | 50 | 37 | 42 | 24 | 19 |
| Musta | 6 | 6 | 4 | 1 | 7 | 8 | 9 | 13 | 17 | 14 | 15 | 9 |
| Gargur | 20 | 4 | 5 | 9 | 4 | 11 | 11 | 18 | 19 | 34 | 44 | 12 |
| Mellieha | — | — | 1 | — | — | 2 | 2 | 5 | 6 | 5 | 8 | 2 |
| Luca | 1 | 1 | — | 1 | 1 | 2 | — | 4 | 2 | 6 | 6 | 2 |
| Zurricco | 10 | — | 2 | 4 | 3 | 5 | 12 | 6 | 3 | 2 | 4 | 5 |
| Krendi | — | 4 | 1 | 2 | 4 | 1 | 4 | 6 | 3 | 2 | 5 | 13 |
| Safi | — | — | — | — | — | — | — | — | — | 1 | 2 | 6 |
| Micabiba | 1 | 3 | — | — | 1 | 1 | 1 | 5 | — | 1 | 1 | — |
| Chircop | 1 | 1 | — | — | 1 | 1 | 2 | 3 | 3 | 3 | 2 | 5 |
| Zeitun | — | 1 | — | — | — | — | — | — | 2 | 2 | — | 2 |
| Axiak | 13 | 10 | 13 | 13 | 24 | 10 | 32 | 31 | 26 | 9 | 25 | 16 |
| Għadira | 4 | 1 | 1 | 1 | 1 | 4 | 3 | 3 | 8 | 1 | 3 | 3 |
| Għadira | 3 | — | 2 | 1 | 1 | 4 | 8 | 4 | 2 | 2 | 1 | 2 |
| | 104 | 61 | 70 | 100 | 131 | 107 | 221 | 240 | 219 | 212 | 188 | 160 |

I take March to September inclusive as being the driest and consequently the most dusty months; and January, February, October, November, and December as the wettest and least dusty months. Allowing a month for incubation and notification, we have April to October representing the dry part of the year and the remaining months the wet.

In the urban area the average number of cases in the dry season was 10.73 per month, and in the wet season 6.16; that is in the proportion of 100 to 56.

In like manner in the suburban area the average number of cases per month in the dry season bears to the average number of cases per month in the wet season the proportion of 100 to 63, while a similar comparison in the rural area gives a proportion of 100 to 66.

| | Proportion of cases per month in the wet season to the cases per month in the dry season. |
|---------------------|---|
| Urban area..... | 100 to 56 |
| Suburban area | 100 „ 63 |
| Rural area | 100 „ 66 |

This is not the result to be expected had contaminated dust contributed largely to the spread of Mediterranean Fever. There is less dust in the urban area, and less opportunity for dust contamination, on account of superior paving, draining, and scavenging, and also because all the urban area abuts upon the sea, much of it being built on tongues of land almost surrounded by water. It would be expected that the difference between the prevalence of Mediterranean Fever in the dusty season and in the wet season would prove least marked in the urban area, but, on the contrary, the difference is most marked in this area. These figures, taken for what they are worth, do not indicate that contaminated dust in the open air has a marked influence upon the incidence of Mediterranean Fever.

In the consideration of excretal infection by way of the hands or by way of food, special incidence upon certain hospital nurses and orderlies who have the handling and cleansing of Mediterranean Fever patients has already been noticed under the heading "Direct Personal Infection."

Abundant opportunity for soiling the hands and for pollution of the food is afforded by the methods of excreta disposal in use in the islands, which have already been referred to in Part I of this report. Amongst 100 houses examined in which Mediterranean Fever had occurred during 1904, I found that 75 had faults of one kind or another which rendered pollution of the hands, or of food, with excretal matter, probable. Amongst 40 other houses not infected with

Mediterranean Fever during 1904, but examined by way of control, I found 55 per cent. suffering from faults of a like kind. The control houses were selected by reason of their similarity to the infected houses; they were very often next door, and they were always of the same class and in the same neighbourhood as the infected houses. Large figures, however, extending over several years, would be required to give value to such data as these. Such as it is, however, the evidence is in favour of the probability of excretal pollution of the hands or food, or of dust inside houses having played a part in the spread of infection. As against this probability there is the fact that amongst the civil population, where opportunity for this kind of infection is far greater than amongst the garrison or Navy, the case incidence of Mediterranean Fever is in general about one-eighth as severe. Here, however, the notification returns are probably at fault.

(3) *Newly-turned earth* has been suspected by more than one observer to be a cause of outbreaks of Mediterranean Fever. When the porous nature of the rock in Malta is considered together with the fact that sewage has been allowed to percolate into it, and into the soil above it, for centuries, it does not seem remarkable that digging operations should have been suspected.

Mr. Cartwright-Read, the Admiralty Superintendent of Works, kindly undertook to furnish me with immediate notice of cases of sickness arising amongst the men employed by him on digging operations in Fort St. Angelo during my stay in Malta. In July 255 men were employed, in August 327, and in September 337. All men absent from work on account of illness during these three months were visited and reported upon, and no case of Mediterranean Fever was discovered. The ground opened up was probably at one time very much fouled, part of it having been the ancient prison of the galley slaves employed by the Knights of St. John.

The Honourable Mr. Gatt, Superintendent of Public Works to the Maltese Government, kindly placed similar facilities at my disposal with regard to gangs of men at work laying sewers during July, August, and September of 1904. The numbers of men employed were 200, 230, and 310, in each month respectively. No case of Mediterranean Fever was detected amongst them.

It appeared to me possible that the men at work in sewer laying had attained a certain immunity from infection, such as is said to be acquired by sewer men at home, and that the opening of the earth might have had a deleterious effect upon the health of the occupants of the houses in the localities where the sewers were being laid.

Curmi, Misida, and Sliema have been sewered during the last few years, the actual period occupied in laying sewers being for Curmi, March, 1901, to October, 1901; for Misida, the whole of 1903; and

for Sliema, November, 1901, to October, 1902. Contrasted with the five-year period 1899 to 1903 there was, as shown below, a slightly greater incidence of Mediterranean Fever in Sliema, and a considerably smaller incidence in Misida during the period that sewers were being laid. In Curmi the incidence was about three times as severe during the laying of the sewers as it was during the five-year period, but here we are dealing with only a very small number of cases. On the whole, the figures below do not indicate that opening streets to lay sewers has any marked effect in increasing the prevalence of Mediterranean Fever.

Table IX.

| | I. Period during which work was in progress. | II. Average number of cases of Mediterranean Fever per year per 10,000 inhabitants. | |
|--------------|---|--|------------------------------------|
| | | During the period set out in Column I. | During the period 1899—1903. |
| Sliema | Nov., 1901—Oct., 1902 | 46·9 | 43·3 |
| Misida | 1903 | 74·4 | 83·6 |
| Curmi | Mar.—Oct., 1901..... | 27·6 | 9·5 |

(4) *Biting Insects*.—Further investigation is necessary before any definite pronouncement can be made as to the part, if any, taken by biting insects in the spread of Mediterranean Fever. Up to the present little is known as to the life history, distribution, and seasonal habits of even the commoner biting insects found in the Maltese Islands. I have, for instance, evidence that mosquitoes bite in the winter in Malta, but I do not know what kind of mosquitoes do so. The sand fly is very prevalent in parts of Malta during the summer, but there is little information as to his breeding places or his time of flight, or his winter habits.

The researches of Shaw and Gilmour would show that it must be a matter of some difficulty for a biting insect to infect himself from the human subject, seeing the sparse numbers in which the *Micrococcus* has been found in the peripheral circulation, and the small amount of blood the insect is capable of taking. Similarly, the chances would be infinitesimal of an insect carrying even a single *Micrococcus melitensis* mechanically upon his proboscis. On the other hand, it may be said that if certain biting insects, like mosquitoes, were capable of infecting themselves with Mediterranean Fever and of transferring the infection, the disease would be much more prevalent than it is.

The special incidence upon hospital orderlies, in comparison with hospital patients, is against the biting insect theory. The patients would be more likely to get bitten by infected insects than would hospital orderlies, both on account of being constantly in the wards and because they are less able to defend themselves.

(5) *Water* does not appear to have played any considerable part as a carrier of Mediterranean Fever infection in Malta. The public water supply, which is reasonably free from suspicion of contamination, is laid on to every village in Malta except Mellieha, but most householders have an alternative supply in the shape of a rain-water tank, usually open to contamination. Generally speaking, the public water supply is not laid on to the houses, but is fetched from a stand-pipe, and it is apparent that there is often opportunity for contamination in process of transit, or on account of the place where the water is kept. I personally inspected 100 houses in which Mediterranean Fever had occurred during 1904, and as far as I could judge, 35 of them had a water supply that was not reasonably liable to contamination. Out of 40 houses inspected by way of control, 27 per cent. had a water supply which was not reasonably liable to contamination. In the same two groups of houses, 85 of the infected class used the public water service, while only 65 per cent. of the control class used it.

A comparison between the figures relating to enteric fever, often a water borne disease, and the figures relating to Mediterranean Fever, shows little correspondence in the distribution of the two diseases. For example, the average number of cases of enteric fever per 10,000 inhabitants in Malta per year of the period 1894 to 1903 was 7·2, and the incidence on the three areas referred to previously was as follows:—

| | | | | |
|---------------------|-----|------------|-------------|-----------|
| Urban area..... | 7·5 | per 10,000 | inhabitants | per annum |
| Suburban area | 6·6 | | „ | „ |
| Rural area | 6·9 | | „ | „ |

I do not profess to account for this distribution of enteric fever, but it is obviously entirely different from that of Mediterranean Fever.

No connection has ever been demonstrated between any particular branch of the public water supply, nor between any particular well or tank, and an outbreak of Mediterranean Fever, though such connection has frequently been shown with outbreaks of enteric fever in Malta.

Aërated waters are much drunk in the summer time in Malta, but here the question is still one of water. The manufacture is carried on under Government inspection; the water used is generally the public water supply, and in some cases distilled water. No connection has ever been traced between aërated waters and Mediterranean

Fever ; indeed, the majority of rural dwellers who are attacked by Mediterranean Fever seldom take aerated waters. The urban and suburban areas, where aerated waters are most taken, do not show a higher incidence of the disease than the rural area.

(6) *Milk* is not so closely connected with water in Malta as it is in most other countries, because the great majority of people get their milk supply in their own vessels direct from the goat.

My inquiries as to the precise source of milk supply in the houses I visited seldom elicited definite information ; many of the persons interrogated did not know whose goats supplied them, being in the habit of hailing the first goat herd who passed the door.

More particular inquiries as to sources of milk supply in relation with Mediterranean Fever are now being made immediately upon notification, and when the results are tabulated at the end of July, 1905, some definite pronouncement may be possible.

(7) *Uncooked Foods*.—Inquiry was made in a large number of cases with regard to fruits and vegetables, or salads, but nothing tending to incriminate these articles of diet was elicited.

(8) *Infection by Cuts or Abrasions*.—No connection was established between breaches of continuity in the skin and subsequent attacks of Mediterranean Fever. Practically none of the cases I saw had a history of cuts or other abrasions. The only evidence that seems to point in the direction of infection of this kind is the severe incidence of Mediterranean Fever upon patients in the operation ward of the Royal Naval Hospital, Bighi, during 1904, a point which requires further investigation.

The following table gives some idea of the arrangement of the Royal Naval Hospital at Bighi, and the number of patients who developed Mediterranean Fever during 1904, more than 14 days after admission, or less than 14 days after discharge from the hospital.

The chief contributory wards were : C, the operation ward, and D, one of the suppuration wards. Cases were transferred from C to D, if suppuration supervened, and cases were also transferred, when necessary, from D to C. During 1904 seven cases were transferred from C to D, and five from D to C, so that the two wards were in a measure connected with one another. Only one case occurred in E3 or E4, which adjoin the Mediterranean Fever wards and open into them.

| | Jan. | | Feb. | | Mar. | | Apr. | | May. | | June. | | July. | | Aug. | | Sept. | | Oct. | | Nov. | | Dec. | | Total. | | |
|----------------------------------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|-------------|----------|--|
| | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | Admissions. | Attacks. | |
| SURGICAL. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| North block— | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| A (basement), venereal | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C (1st floor), operations | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| E (2nd floor), suppuration | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| South block— | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B (basement), venereal | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| B2 (basement), ophthalmic | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| D (1st floor), suppuration | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| F (2nd floor), suppuration | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Officers' cabins | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| MEDICAL. | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| East block— | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1. Mediterranean Fever | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2. Mediterranean Fever | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3. Enteric and scabies | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4. Zymoties | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Officers' cabins | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| West block— | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 1. General medical | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 2. General medical | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 3. Tubercle | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 4. Tubercle | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Officers' cabins | | | | | | | | | | | | | | | | | | | | | | | | | | | |

PART III.—OUTBREAK OF MEDITERRANEAN FEVER IN THE 2ND
BATTALION OF THE ESSEX REGIMENT.

This was the only definite outbreak of Mediterranean Fever that I had an opportunity to observe personally, during my stay in Malta.

The 2nd Battalion of the Essex Regiment arrived at Malta from England on April 29, 1904. With the exception of some details sent to Gozo, the regiment was quartered at Lower St. Elmo Barracks, in Valetta until May 19th, when it went to Pembroke Camp for musketry. It remained at Pembroke Camp until May 28th, and then went on to the camp at Mellieha, whence it returned to Lower St. Elmo on June 10th. It was still at the last-named barracks at the end of September.

The first known cases of Mediterranean Fever in the battalion occurred on June 10th, when two sergeants fell ill, another went sick on the 11th, a corporal and a sergeant on the 14th, and so the cases kept dropping in until, on August 27th, there had been 35 cases, 21 of which were directly connected with the sergeants' mess.

The type of the disease was very severe. Cases 4, 9, and 13 died and others were still in danger at the end of September.

Table XI shows the number of known cases of Mediterranean Fever in the Essex Regiment and the date of onset of the disease in each instance.

Of these 35 cases, occurring in $2\frac{1}{2}$ months, 21 were directly connected with the sergeants' mess; as regards the remaining 14, indicated in the table by a star, there does not appear to have been such connection except in the case of No. 12A. This man, however, returned to Gozo on June 10 to find his wife and child sickening with Mediterranean Fever. The probability of his having been infected by his wife or child, or from the same source as they were, seems stronger than the probability of his having been infected while with the regiment. I have therefore not included him amongst the cases likely to have been infected at the sergeants' mess.

Case 20 was in hospital with simple continued fever during most of the regiment's stay at Pembroke, and he returned to duty at St. Elmo instead of going to Mellieha. He left St. Elmo before the regiment's return, and went to Ghain Tuffieha Camp, and from there to Gozo, where he was attacked by Mediterranean Fever. His illness has therefore no causal relation with the main part of the regiment.

The 21 cases connected with the sergeants' mess form a continuous series of 19 attacks in six weeks, with never more than three or four days' interval. After July 16 there is an interval of 11 days

Table XI.

| No. | Date of onset. | Name. | Remarks. |
|-------|----------------|----------------------|---|
| 1 | June 10 | Sergt. H. | |
| 2.. | " 10 | " B. | |
| 3.. | " 11 | " W. | |
| 4.. | " 14 | Lance-Sergt. H. | |
| 5.. | " 14 | Corpl. J. | Used to go into sergeants' mess for orders. |
| 6.. | " 17 | Sergt. L. | |
| 7.. | " 20 | Pte. M. | Waiter in sergeants' mess. |
| * 8.. | " 21 | " H. | No connection shown with sergeants' mess. |
| 9.. | " 23 | Sergt. F. | |
| 10.. | " 23 | Lance-Sergt. S. | |
| 11.. | " 24 | " B. | |
| 12.. | " 25 | " B. | |
| *12A | " 25 | " W. | Wife and child had Mediterranean Fever at Gozo, where he rejoined them June 10. |
| 13.. | " 28 | " Y. | |
| *14.. | " 29 | Pte. F. | No connection shown with sergeants' mess. |
| 15.. | July 1 | Sergt. P. | |
| 16.. | " 1 | " F. | |
| 17.. | " 4 | " M. | |
| 18.. | " 5 | " V. | |
| 19.. | " 7 | " K. | |
| *20.. | " 8 | Pte. P. | |
| 21.. | " 12 | " M. | Waiter " in sergeants' mess " until May 28, 1904. |
| *22.. | " 15 | " L. | No connection shown with sergeants' mess. |
| 23.. | " 16 | Lance-Corpl. B. | Cleaned men's latrines; was often in sergeants' mess for odd jobs. |
| *24.. | " 23 | Pte. H. | No connection shown with sergeants' mess. |
| 25.. | " 27 | " L. | Waiter in sergeants' mess. |
| 26.. | " 31 | Sergt. H. | |
| *27.. | Aug. 5 | Lance-Corpl. S. | No connection shown with sergeants' mess. |
| *28.. | " 10 | Pte. W. | " " " " |
| *29.. | " 13 | " C. | " " " " |
| *30.. | " 13 | " C. | " " " " |
| *31.. | " 14 | " C. | " " " " |
| *32.. | " 18 | " S. | " " " " |
| *33.. | " 26 | " A. | " " " " |
| *34.. | " 27 | " W. | " " " " |

without a fresh case, and then follows Case 25 on July 27, and four days later, on the 31st, Case 26. There were no other known cases among persons connected with the sergeants' mess up to the end of September, when I left Malta.

As nearly as could be ascertained there were about 60 persons connected with the sergeants' mess, while there were in all 616 men

and officers at Mellieha. Of the 60 persons, 21 were attacked by Mediterranean Fever, while of the remaining 556 persons, 14 only were attacked, and these for the most part at a later period than members of the mess in question.

A short description of the three places occupied by the regiment between April 29, the date of their arrival from England, and the end of September, is as follows:—

Pembroke Camp is the chief musketry camp for the island. It is situated a few miles north of Valetta on the sea shore. The camp is on rocky ground sloping to the sea. The rock is the common calcareous sandstone of Malta, and the intervals between the outcrops are filled by a sandy red loam, which easily pulverises and forms dust.

Mellieha Camp is situated on the bay of the same name, about half-a-mile north of the village of Mellieha, the most northerly village in Malta. The soil in and around the camp is loose and sandy, overlying the upper coralline limestone. The sergeants' mess, a wooden building, is on the edge of the camp, just above a steep sandy slope leading down to the sea.

Lower St. Elmo is a part of the fort which occupies the seaward end of the tongue of land on which Valetta is built. The barracks are below the level of the outer ramparts. The fort is built entirely on the calcareous sandstone.

Both at Pembroke and at Mellieha the regiment was under canvas. An examination of the situation of the tents in which persons attacked by Mediterranean Fever had slept, showed no considerable incidence on any one part of the camp more than on another.

At Pembroke the sergeants messed in a marquee which was equally exposed to the dust with the tents in which the privates lived and ate their food. The marquee stood upon similar soil to the tents, and it was subject to the same conditions with regard to proximity to latrines and urine tubs. It was no more liable to foul air emanations than the tents of the privates. At Pembroke the same latrines (dry earth) were used by the sergeants and by the privates, though a portion was reserved for the former. Complaints were made of offensive smell and of flies at the latrines.

At Mellieha the sergeants' mess hut stood some 40 yards away from the tents of the men. It was a wooden building, raised from the ground on posts, and protected from dust by windows. Built on to it were two water-closets, which were not used during the regiment's stay, because they were said to be out of order. These closets were directly connected with the sergeants' mess room by a louvred ventilator. I found after inquiry on the spot, that these water-closets had become unsealed owing to evaporation. It was therefore possible for sewer air to escape into the mess room from the whole length of sewer below the closets, and from the septic tank to which

this sewer conveyed the sewage of the camp. There were separate latrines (water carriage) for the sergeants and the privates at Mellieha; no complaint of smell or nuisance was made as regards either set of latrines.

At St. Elmo the sergeants' mess was situated on a site elevated some 30 feet above the barrack yard across which it faced the men's barrack rooms, at a distance of 40 or 50 yards. The mess was approached by a path seldom used for any other purpose, and the latrines were on the same level as the mess room, about 10 yards distant from it. I found no fault with these latrines other than the faults inherent to their pattern, which I have already discussed; but the latrines for privates in the same barracks were insufficiently flushed and most offensive.

No duty could be heard of which was likely to bring the sergeants of the regiment together into one place, except that of marking at the butts. (There were butts at Pembroke, but not at Mellieha, or St. Elmo.) There were, however, as many corporals and privates employed at the butts as sergeants, yet there was no special incidence of Mediterranean Fever on the former as there was on the latter.

The only places frequented by sergeants, but not by privates, were, at Mellieha and St. Elmo, the sergeants' mess and latrines, at Pembroke, and the sergeants' mess.

The sergeants who were attacked by Mediterranean Fever seem to have been infected roughly in proportion to the amount of time spent in the mess. The married sergeants would frequent the mess at Pembroke and Mellieha equally with the unmarried ones, while at St. Elmo they would spend more of their time at the married quarters. Twenty out of 51 sergeants who frequented the mess at all three places were married. Six of them were attacked before June 25, of whom three were married (the presumption being that cases before the 25th were infected at Mellieha or Pembroke), while of the remaining 12 cases, infected presumably at St. Elmo, only three were married. The number of cases before the 25th is, however, too small to allow of any great importance being attached to the increased incidence on married sergeants before that date.

Supposing, however, that the sergeants' mess, including, at Mellieha and St. Elmo, the sergeants' latrines, afforded the conditions for contracting infection, the question arises as to whether the mess at Pembroke, that at Mellieha, or that at St. Elmo, was chiefly concerned.

The first cases occurred on the 14th day after the regiment left Pembroke. This allows time for an incubation period consistent with the supposition that the disease was contracted at Mellieha, and to a corresponding extent tends to exculpate Pembroke, though it remains possible, of course, that the first few cases became infected at Pembroke. At Mellieha there were circumstances which differentiated the condi-

tions of life at the sergeants' mess from the conditions obtaining in the camp generally, but at Pembroke the same kind of difference did not exist. Some such differences as those obtaining at Mellieha deserve attention in attempting to account for the enormous excess of incidence upon persons connected with the sergeants' mess. Upon the whole, I think it is not likely that the sergeants' mess at Pembroke was seriously, if at all, concerned in the spread of the fever in the regiment, and that it is likely that the cause of the outbreak operated first of all at Mellieha. It would appear certain, however, that the sergeants' mess at St. Elmo had later on a share in spreading the infection, because if we were to suppose that infection was spread only at Mellieha, the average incubation period for Cases 13, 15, 16, 17, 18, and 19, would amount to at least 23 days, and to much more if Cases 21, 23, 25, and 26, were also referred to Mellieha. Although very long incubation periods have occasionally been reported, yet a succession of cases having so lengthened an incubation as the above, seems to me very improbable. I refer here only to the cases connected with the sergeants' mess, the other 13 cases occurring as they did most of them later on, and amongst over 500 men, did not show an incidence much greater than is to be expected under ordinary circumstances in Malta.

Food and Drink.—Bread, water, and milk came to sergeants and privates from the same sources. Butter, fruit, vegetables (salads, tomatoes, etc.), were eaten by both. Mineral waters were drunk by both, but those drunk by the sergeants were said to be made from distilled water, while those supplied to privates were not.

More precise inquiries were made amongst the members of the sergeants' mess and persons connected with it, including those of them who were ill with Mediterranean Fever.

Water.—Inquiry was made of 52 men connected with the sergeants' mess, including 19 of those attacked by the fever, as to water drinking. Cases 5 and 23 were not included because they neither ate nor drank in the sergeants' mess. Thirty-one men never drank water, and one other man only drank it on one occasion, and may for practical purposes be considered a non-water drinker. Of these 32, 11 were attacked (34·4 per cent.). Eleven men seldom drank it, and seven of these were attacked (63·6 per cent.). Nine men habitually drank water, and one was attacked (11·1 per cent.). Non-water drinkers then were attacked at the rate of 34 per cent., while water drinkers (habitual and occasional) were attacked at the rate of 40 per cent. On the other hand, occasional water drinkers were attacked nearly six times as severely as habitual water drinkers. The 11 occasional water drinkers drank it only when employed at the butts, when they obtained it from their water bottles. The habitual water drinkers also drank at the butts, in the same way, water from a like source.

Milk.—As result of inquiry made of 58 men connected with the sergeants' mess, it appeared that 3 drank unboiled milk by itself, and that 1 of them was attacked (33·3 per cent.); while of 55 men who did not drink unboiled milk by itself, 17 were attacked (30·9 per cent.). Case 4 was too ill to be interrogated, and is consequently excluded.

Milk with tea, coffee, or cocoa, was taken by all except three men. None of these three were attacked.

Mineral Waters.—Two out of 59 men interrogated did not drink mineral waters. One of these two was attacked.

Beer.—Of 59 men interrogated, it was ascertained that 52 took beer, and 17 of them were attacked (32·7 per cent.); while of 7 that did not take beer, 2 were attacked (28·6 per cent.).

Raw Vegetables (tomatoes, salads, etc.) were eaten habitually by 42 men out of 59, seldom by 9 men, and never by 8 men. Of the 51 who ate raw vegetables 14 were attacked (27·5 per cent.), while of the 8 men who did not eat them 5 were attacked (62·5 per cent.). Of the 14 cases among eaters of raw vegetables, 12 occurred among the habitual consumers and 2 among the occasional consumers.

Fruit.—Uncooked fruit was eaten habitually by 38 men out of 58, seldom by 13 men, and never by 7 men. Ten of the 38 who eat fruit habitually were attacked (26·3 per cent.). Four of the 13 men who seldom eat fruit were attacked (30·8 per cent.), and 5 out of the 7 who never ate it were attacked (71·1 per cent.).

Bread and Butter were partaken of by all from a like source.

It cannot be said that any one of the foods or drinks inquired about is incriminated by the above details, nor can there be much doubt that if any of the foods or drinks had been largely concerned in spreading the disease, the fact would have appeared. There is a slightly greater incidence upon those who drank water, but not sufficient, in face of the small numbers dealt with, to found any conclusion upon.

Bathing.—Inquiry was made as to bathing, because it has frequently been suggested that Mediterranean Fever might be caused by bathing in sewage-polluted waters. At Mellicha the sewage from the camp is discharged into the shallow waters of the bay, after passing through a septic tank, and at St. Elmo, situated as it is on the point separating the Grand and Quarantine harbours, there is considerable pollution of the water by sewage. Out of 64 men interrogated, 58 bathed and 19 were attacked (32·8 per cent.), while of 6 men who did not bathe, 2 were attacked (33·3 per cent.).

Personal Infection.—The question of direct personal infection from man to man was considered. This may have occurred in the mess-room, but if so, why did it not also occur outside from sergeants to men; unless, indeed, it happened that infection was largely conveyed from man to man by means of spray thrown into the air in the act of speaking or coughing. This method of infection would no doubt be favoured

by the still air of the mess room, and by the propinquity therein of the sergeants to one another during conversation; it would not be so likely to operate in the open air, nor perhaps in the tents or barrack rooms at night. If direct personal infection, other than through saliva, were the mode of infection in this outbreak, it would have better opportunity for taking effect in the tents and barrack rooms at night than in the mess room. Many of those attacked by Mediterranean Fever slept in tents with other persons not connected with the sergeants' mess. For instance, 7 privates slept in the tent with Case 12 at Mellieha, and 21 privates in the same room with him at St. Elmo. Most of the unmarried sergeants who fell ill at St. Elmo slept in bunks in the room with 20 or more men, yet none of these men were infected. Five cases altogether occurred in persons who had slept in the same tent or room with earlier cases, and 3 of the 5 were connected with the sergeants' mess.

Biting Insects.—In view of the theory put forward by Zammit, inquiry was made of 58 men connected with the sergeants' mess as to whether they had been bitten by mosquitoes or sand flies. It was considered at the time that the answers received would probably be a better index of the toughness or insensibility of the deponent's skin, than of the facts as they really were; nevertheless the results are given for what they are worth. Forty-three men were conscious of having been bitten, and 7 of them were attacked by Mediterranean Fever (16·3 per cent.); while of 19 who were not aware of having been bitten, 11 were attacked (57·9 per cent.). These figures may be claimed as unfavourable to the hypothesis that biting insects play a part in the transmission of Mediterranean Fever to man. On the other hand, it may be contended that those who were not aware of having been bitten had the more insensible skins, and hence were less likely to take precautions with a view to preventing the insects biting them, and, in consequence, were the more likely to have been bitten. Cases 4, 9, and 13 were too ill to answer this interrogation, and are consequently excluded.

If infection were conveyed by a biting insect, the insect, to fit in with the circumstances of this outbreak, would require to be one which did not bite in bright sunlight, nor in the dark. It was practically only in bright sunlight or in the dark that the sergeants mixed with the men, and if the insect were in the habit of biting under these conditions, the men would have been infected as well as the sergeants. Again, the insect must be one likely to confine itself strictly to one building or marquee, seldom or never wandering 50 yards away, for at Pembroke, Mellieha, and St. Elmo the tents or barrack rooms were within 50 yards of the sergeants' mess.

It may be asked how would the insect be likely to have become infected. Mellieha Camp was occupied from May 3 to June 9 by three different regiments, but no case of Mediterranean Fever is

known to have occurred amongst them during their stay or 14 days after. Neither were any cases reported from Mellieha village during this period. The possibility, however, of there having been unrecognised cases of Mediterranean Fever in one of these regiments, or in the Essex Regiment, cannot be disregarded.

It should be noted that non-commissioned officers would provide better opportunity for the spread of personal or insect-borne infection, than would private soldiers. The former are always loth to go to hospital, and usually defer reporting themselves sick until the last possible moment, while the latter generally report at once. In this outbreak, for instance, Case 6 did not go into hospital until July 15, although he became ill on June 17.

The only biting insect, of which I am aware, that comes at all near fulfilling the conditions which this outbreak seems to require is the female *Stegomyia fasciata*. There is, however, at present, wide divergence in the views of various observers as to her flight and habits of biting. It would be remarkable if so short a distance as 50 yards proved an insuperable barrier for a winged insect, even for one which, like the *Stegomyia*, is generally supposed not to wander far. Specimens of the *Stegomyia* were to be found at the time at Mellieha and St. Elmo, but they were also to be found at Valetta Station Hospital into which all the cases were removed. In this hospital Mediterranean Fever patients were separated from other patients only by a partition 9 or 10 feet high, in a ward more than 20 feet high, and mosquito nets were not in general use, and yet it does not appear that other patients became infected specially at that time.

Latrines in Connection with the Outbreak.—In view of Horrocks' discovery that the *Micrococcus melitensis* is excreted in the urine, and the possibility that it is also excreted in the fæces, the question arises, supposing that infection were spread by the sergeants' mess at Mellieha and St. Elmo, was it spread principally by way of the mess room, or by the latrines? The latter, it will be remembered, were separate from those of the privates both at Mellieha and at St. Elmo. I am not now considering the disused water-closets at Mellieha. Both the latrines and the mess room were used by everybody connected with the sergeants' mess, except Cases 5 and 23, so that it is difficult to find any evidence to incriminate the one place as against the other. It can be said, however, that it is difficult to imagine an infection inherent to the mess room yet not likely to be conveyed directly from man to man outside, while if the infection be supposed to be inherent to the latrines and of excretal origin, the difficulty is not nearly so great: infected dust, due to the pattern of the latrines, or infection of the hands and thence the mouth or nose for instance. Against the possibility of the latrines having been principally concerned in the spread of infection is the following negative evidence

which is not of sufficient weight to be at all conclusive:—One and the same man attended to the flushing and cleansing of the sergeants' latrines both at Mellieha and St. Elmo, and he was not attacked by Mediterranean Fever. Cases 5 and 23 did not use the sergeants' latrines. In addition it may be said that if the first few cases of the outbreak were infected at Pembroke, where the sergeants used a portion of the same latrines as the men, it would be expected that some of the men would have been infected if the latrines were the source of infection. I have, however, already said that I do not think it probable that the early cases were infected at Pembroke, and the man who attended to the sergeants' latrines may have been immune to Mediterranean Fever.*

With regard to the two water-closets through which it was possible that sewer air obtained access to the sergeants' mess at Mellieha, there appeared to be two ways in which they might have contributed to spread the fever: either by allowing infected dust to enter the mess room, or by allowing sewer air to enter, and thus weakening the natural tissue resistance by causing sore throat, or general loss of tone. None of those attacked by the fever, however, complained of sore throat previous to the onset of the fever. As to the infected dust theory, specimens were procured of the dust in the ventilator and on the closet pan, and were injected into monkeys by Horrocks without producing any ill effect. These specimens were, however, very minute, and were procured some weeks after the regiment left Mellieha. But supposing that infected dust from these closets, or from the latrines, were the cause of fever at Mellieha, what then caused the continuance of the outbreak at St. Elmo? Again, if the general tone of the men's health was lowered by the inhalation of sewer air in the mess room at Mellieha, and they were thus rendered specially liable to Mediterranean Fever, their health should have recovered its normal tone at St. Elmo, other conditions being equal, and the outbreak should have ceased, which was not the case.

Septic Infection.—Inquiry was made of those attacked as to whether they had suffered from cuts or boils, or other skin lesions, before the onset of the fever, but in no case was the reply in the affirmative.

Conclusion.—The available evidence is not such as to justify a definite

* "Carbolic" powder was used as a disinfectant in the latrines from the time of the regiment's arrival in Malta until May 18, when it was discontinued by order of the War Office, on the grounds that it was not a disinfectant but only a deodorant. Its use was resumed by the Essex Regiment on June 20. In view of any question arising as to a connection between the disuse of the "carbolic" powder and the outbreak of fever, a specimen of the powder was examined for me by Horrocks, and he found that a 10-per-cent. solution in urine failed to kill a culture of *M. melitensis* in one hour. I do not therefore think that the use, or disuse of the powder can have had any influence on the potentialities of the latrines to spread infection.

conclusion as to the manner of propagation of Mediterranean Fever in this outbreak.

The facts narrated, however, are not without value for the epidemiologist. If they cannot be held to warrant positive assertion of the transmission of the malady by a particular agency, they are at least of service in strongly suggesting the exclusion, in this instance, of certain possible factors, and as regards other such factors, in affording means of considering the relative degrees of probability of their having been concerned with the incidence of the fever.

Regarded in this light, the evidence may fairly be held to indicate that articles of food and drink played no appreciable part in the dissemination of the disease. A like inference is justified concerning the possible influence of conditions associated with bathing, and with inhalation or swallowing of infected dust in the open.

The possibility of the fever having been conveyed by biting insects cannot so readily be dismissed. But a careful review of the facts and conditions, adduced under this head does not favour acceptance of the hypothesis that the explanation of this outbreak is to be found in this direction.

The facts reviewed in this report under the heading of direct personal infection are only such as would suggest the transmission of the fever by this agency if the manner of transmission in this instance had been such as almost entirely to limit its operation to the sergeants' mess. I am not, so far, in possession of any facts on the bacteriological side capable of strengthening, or negating the possibility of transmission of the fever by saliva. I do not know even that the *Micrococcus melitensis* exists in the saliva of patients. It may be said, however, that infection by means of saliva affords a solution of the problem of transmission not inconsistent with the facts in this outbreak, so far as I have been able to discover them; but it must be added that other facts noticed in Part II of this report under the heading "Direct Personal Infection," do not point to the probability of saliva spray having played a part in the spread of infection. (See p. 37 *et seq.*)

There remain for consideration the possibilities of the fever having been transmitted by conditions other than direct personal infection, or conveyance of the disease by biting insects, associated with the latrines of the sergeants' mess, or with the sergeants' mess itself.

Hypothesis that Mediterranean Fever may be a "filth disease," and that the latrines in question became and remained for some time infected by *Micrococcus melitensis*, passed in the urine or fæces of persons using them, would point rather to the latrines having had relation with propagation of the disease than to like relation of the mess itself. Such hypothesis, however, involves considerations that require further investigation, and, without more complete knowledge than is now available, can be no more than tentative.

Besides the condition suggested by this hypothesis, there may be others, at present unknown, which future epidemiological investigation, combined with further acquaintance with the life history and habits of the specific contagion of the malady, may serve to reveal, and which may be found to explain, as regards this outbreak of Mediterranean Fever, the special incidence of the disease upon persons frequenting the sergeants' mess or using their latrines.

These inferences, and the relative degree of probability of each, have relation solely to this particular outbreak of Mediterranean Fever. Even did the evidence point conclusively to one particular agency as being solely concerned with the outbreak, it would not necessarily follow therefrom that such agency would have to be regarded as the only one having concern with the transmission of Mediterranean Fever generally.

PART IV.—GENERAL SUMMARY AND CONCLUSION.

Hampered by a sense of the inaccuracy of the civil notification returns, I have only attempted to draw the most general conclusions from them, except with regard to the seasonal incidence.

The evidence I have been able to collect is not sufficient to lead to any final conclusion. I hope, however, that I have been able to indicate in the course of Part II some directions in which further epidemiological investigations would be likely to prove profitable.

The distribution of Mediterranean Fever amongst the civil population goes to show that, outside certain paved and drained areas, aggregation of persons in one locality, and density of population upon area in a district, favour the spread of the disease. The distribution amongst the garrison depends mainly on the age of the men and their length of service in Malta, new arrivals and young men being more frequently attacked. As regards the Navy, I have only been able to obtain figures for three years. So far as they go, they tend to show that, when a ship is invaded in one year, it is also invaded in each successive year, if it remain on the station.

The incubation period seems, on the data I have been able to collect, to be about 14 days, but further evidence is necessary before a definite conclusion can be reached.

As to the mode of entry of the specific infection into the human body, the facts do not permit of a definite pronouncement. The evidence, so far as it goes, seems to show that food and drink have no marked connection with the spread of the fever. Newly turned earth falls into a like category.

As a whole, the facts do not indicate that dust infection, outside dwellings, or direct personal infection by contact, breath, or saliva, plays an important part in spread of the disease, but there is not evidence to justify the exclusion of any of these factors.

I have been able to collect little evidence either for or against the carriage of infection by biting insects.

The facts with regard to infection by means of excretal pollution of the hands, the food or the dust in houses, so far as I have been able to deal with them, are suspicious, but they are not sufficiently strong to justify any conclusion.

Some reform of the notification system in Malta is necessary before epidemiological investigation can be expected to produce the best results. In addition, facts must be collected and recorded immediately after their occurrence by competent observers. Such work cannot be adequately performed by the sanitary inspectors as at present trained in Malta.

I have endeavoured to provide for the immediate record of a certain number of facts in relation to cases of Mediterranean Fever during the year ending July 31, 1905, amongst the civil population, and in the Services. With regard to the former, I fear that laxity of notification will prove a stumbling block. I regret that an urgent invitation to the Maltese medical men to forward blood samples to the public health laboratory for confirmation, did not meet with the response I expected.

I hope, however, that the facts now being recorded may prove useful in the consideration of some points.

In the meantime, I am still in process of receiving information from Malta which I have requested, as I found it necessary, and I should prefer to await its arrival and the consideration of the facts for the year ending July 31, 1905, before making any recommendations.

I have to thank the following gentlemen for much help and information given me in the course of this inquiry :—Deputy-Inspector Cox, R.N.; Fleet-Surgeon Bassett-Smith, R.N.; Staff-Surgeon Gilmour, R.N.; Colonel Wolesley, R.A.M.C.; Lieutenant-Colonel Rhodes, R.A.M.C.; Captain Kennedy, R.A.M.C.; Lieutenant-Colonel Adair, R.E.; Lieutenant-Colonel Winter, Director of Supply and Transport; and Major Boyce, D.S.O.; the Honourable A. Gatt, Superintendent of Public Works; the Honourable A. Micallef, Comptroller of Charitable Institutions; Mr. Cartwright-Reed, Admiralty Superintendent of Works; the Rev. Father Dobson, S.J., and the medical officers of health of Malta and Gozo.

ON THE SAPROPHYTIC LIFE OF THE *MICROCOCCUS* *MELITENSIS*.

By Fleet-Surgeon P. W. BASSETT-SMITH, R.N.

In compliance with suggestions made at the Sub-Committee Meeting of November 27, 1904, I have, at Haslar, carried out independently, during the last three months, some experiments relating to the vitality of the *M. melitensis* outside the body, with special reference to the infection of the soil, clothing, sea and tap-water, through the agency of infected urine. During this time I have not myself been able to isolate the *M. melitensis* from the urine of any clinical case in the wards (most of them being chronic, with relapses), though it was often present in the peripheral blood, and have, therefore, had to employ urine artificially infected; from the experiments it was apparent that the grosser the infection the longer could the organism be recovered from the material infected.

Some check experiments were also made with broth cultures as a means of infection, though in all cases these had been previously cultured for a certain time in human urine, which apparently did not decrease the vitality of the *M. melitensis*.

In proving the results of these experiments, as to purity of cultures, the following tests were carried out:—

1. Microscopical examination for morphological characters.
2. Alkaline reaction with litmus broth and milk.
3. Inability to stain by Gram.
4. Reaction with known serum of a Mediterranean Fever case.

Vitality of the M. melitensis in Urine.

Series 1.—The urine employed was that taken from Mediterranean Fever cases, which had been proved not to contain the *M. melitensis*, and had been sterilised on three days, but not otherwise treated.

No. 1.—A considerable quantity of a surface culture on agar of *M. melitensis*, originally obtained from Netley, in 1901, was emulsified with 10 c.c. of sterilised urine from a Mediterranean Fever case. It was kept at a temperature of 22° C.

This was subcultured successfully daily up to the 18th, after which it was not recovered.

No. 2.—Equal quantities of a five day old broth culture of *M. melitensis* and sterilised Mediterranean Fever urine, were mixed and subcultured daily on agar. The organisms gradually died out, and were last detected on the 14th day.

No. 3.—Strongly alkaline urine was infected from an agar culture of *M. melitensis* of three days' growth. Here the organisms more quickly died out, being last recovered on the 12th day.

No. 4.—Sterilised urine of a Mediterranean Fever case was infected from an agar culture, which had been previously passed through urine and sea water. In this case it was not recovered from the urine later than the 9th day. The urine was then very alkaline, equal to standard deci-normal soda sol.

No. 5.—Very slightly alkaline sterilised urine of a Mediterranean Fever case was infected from an agar culture, which had been previously passed through tap-water and urine.

In this the *M. melitensis* was regularly recovered, growing normally up to 41 days, the urine infected remaining perfectly clear, and was only slightly alkaline when the organism died out.

No. 6.—Faintly alkaline sterilised urine, rich in urates, of a case in the ward was infected by an agar culture derived from a sea-water one, inoculated from an artificially infected urine.

In this the *M. melitensis* again retained vitality for an exceptionally long period, viz., 39 days, the last subculture reacting normally in all respects.

Viability in Sea-Water.

Series 2.—These were made with ordinary sea-water taken from the harbour, sterilised, and, after inoculation, placed in the 22° C. incubator, and evaporation prevented.

No. 1.—Ten c.c. of sterilised sea-water was infected from a five-day old agar culture of *M. melitensis* derived from artificially infected urine. In the subcultures the colonies gradually became fewer, the last being found on the 26th day.

No. 2.—Ten c.c. of sterilised sea-water was infected with 1 c.c. of seven-day old broth culture of *M. melitensis*, which had been derived from artificially infected urine. From this the last successful subculture was made on the 21st day, when the tube was accidentally broken.

No. 3.—Ten c.c. of sterilised sea-water was infected with 1 c.c. of slightly alkaline urine strongly infected with *M. melitensis* derived from artificially infected urine, that is, two passages through urine; subcultures gave abundant growth until 30 days, when it appeared to die out rapidly, the last being obtained on the 34th day.

Viability in Tap-Water.

Series 3.—These were made from sterilised tap-water of the laboratory after inoculation, being kept in 22° C. incubator.

No. 1.—Ten c.c. of sterilised tap-water was infected from an agar

culture of *M. melitensis* derived from artificially infected urine. The last successful subculture was obtained on the 23rd day.

No. 2.—Ten c.c. of sterilised tap-water was infected with 1 c.c. of a seven-day old broth culture of *M. melitensis* derived from artificially infected urine. Growth was obtained up to the 18th day, when the tube became contaminated.

No. 3.—Ten c.c. of sterilised tap-water was inoculated with 1 c.c. of slightly alkaline urine freshly infected with *M. melitensis* which had been grown in urine. Growth was obtained abundantly until the 26th day, the last being obtained on the 30th.

Viability in Fabric which had been Infected, and Dried in Hot Incubator.

Series 4.—Small squares of flannel fabric were used. These were soaked for certain periods in the culture, drained, and then dried slowly in the hot incubator.

No. 1.—The squares of fabric were immersed for 24 hours in a broth culture of *M. melitensis*, then dried as above stated. One of these was removed every three days, placed in broth, and finally subcultured on agar. Recovered for 37 days.

No. 2.—Immersed for a quarter of an hour in infected urine. Recovered for 7 days only.

No. 3.—Immersed for half an hour in grossly infected urine. Recovered for 15 days.

No. 4.—Squares soaked for 24 hours in urine (Series 1, No. 5) on 10th day of growth. *M. melitensis* recovered for 26 days. The squares soaked became discoloured and stiff from impregnation with urinary constituents.

No. 5.—Squares soaked in infected sea-water for 12 hours. No growth obtained on the 7th day.

Viability in Artificially Infected Dust.

Series 5.—Fine bath brick dust was sterilised, and then soaked for one hour in infected media, drained, and dried in hot incubator. Subcultures were regularly made from this with litmus broth, and finally from this agar cultures, and tested for purity.

No. 1.—Fine oolitic dust infected for one hour with five-day old broth culture, derived from artificially infected urine. *M. melitensis* recovered for 25 days.

No. 2.—As above. *M. melitensis* recovered for 26 days.

No. 3.—Oolitic dust infected with seven-day old broth culture derived from infected urine. Growth abundant and typical. Recovered up to 36 days.

No. 4.—Oolitic dust soaked in slightly alkaline sterilised urine,

grossly infected with *M. melitensis*. Growth was recovered for 30 days.

No. 5.—Oolitic dust soaked in urine in which *M. melitensis* was growing feebly. Not recovered on 3rd day, or any date after.

Series 5A.—Road dust with vegetable and other *débris* collected and thoroughly sterilised, and tested by control cultures. Treated in similar manner to oolitic dust.

No. 1.—Road dust infected by soaking in five-day old broth culture of *M. melitensis* derived from artificially infected urine. Recovered for 44 days.

No. 2.—Road dust soaked in urine strongly infected with *M. melitensis*. Recovered for 16 days.

No. 3.—Road dust soaked in urine in which *M. melitensis* was growing feebly. Not recovered on 3rd day, or any subsequent date.

No. 4.—Road dust infected with urine and broth culture of *M. melitensis* in equal parts. Recovered for 8 days only.

Table of Results of Vitality of the *M. melitensis* outside the Body.

| Medium tested. | Source of supply. | Number of days on which the <i>M. melitensis</i> was recovered. |
|-----------------------------------|------------------------------|---|
| Urine, sterilised. | Agar culture | 9 |
| | " | 12 |
| | " | 18 |
| | " | 39 |
| | " | 41 |
| Sea-water, sterilised | Broth culture | 14 |
| | " | 21 (tube broken) |
| | Agar culture | 26 |
| Tap-water, sterilised. | Infected urine | 34 |
| | Agar culture | 23 |
| | Broth culture. | 18 (contaminated) |
| Fabric, infected and dried.. | Infected urine | 30 |
| | " $\frac{1}{2}$ hour. | 7 |
| | " $\frac{1}{2}$ " | 15 |
| | " 24 hours. | 26 |
| | Broth culture 24 " | 37 |
| Dust, oolitic, infected and dried | Sea-water 12 hours | Less than 7 |
| | Broth culture. | 25 |
| | " | 26 |
| | " | 36 |
| | Infected urine (strong) | 30 |
| Road dust, infected and dried | " (weak) | Under 3 |
| | Broth culture. | 44 |
| | Infected urine (strong) .. | 16 |
| | " (weak) | Under 3 |
| | Urine and broth | 8 |

OBSERVATIONS ON THE VIRULENCE OF *MICROCOCCUS MELITENSIS* FOR THE GUINEA-PIG.

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Summary.

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The Pathogenic Action of M. melitensis.

The degree of virulence possessed by *M. melitensis* for such rodents as the rabbit and guinea-pig is naturally exceedingly low, and in order to produce a fatal infection in these animals, it is necessary to introduce enormous numbers of cocci subcutaneously or intraperitoneally; even then the infection follows a protracted course, and weeks or even months may pass before death takes place.

The majority of the animal experiments by early observers was carried out upon the monkey, an animal which, as originally shown by Bruce,* and afterwards by Hughes, can be easily and certainly infected, chiefly for the reason that attempts to produce fatal infection in the usual laboratory rodents (the rabbit, guinea-pig, and mouse) were for the most part unsuccessful, certainly no appreciable increase in virulence could be demonstrated.

In 1898, however, Durham† applied the method Catani had used to

* Bruce, "Sur une nouvelle forme de fièvre rencontrée sur les bords de la Méditerranée," *Annales de l'Institut Pasteur*, vol. 7, 1893, pp. 289—304; 'Practitioner,' 40, 1888, p. 241; Hughes, "Investigations into the Etiology of Mediterranean Fevers," 'Lancet,' vol. 2, 1892, p. 1265.

† Durham, "Some Observations on the *Micrococcus melitensis*" (of Bruce), 'Journ. of Path. and Bact.,' vol. 5, December, 1898, pp. 377—388.

raise the virulence of the influenza bacillus to *M. melitensis*, and was able to show that the natural resistance of both the rabbit and the guinea-pig was much more readily overcome if the Micrococcus was introduced directly into the brain substance. Further, by a short series of intracerebral passages, he succeeded in slightly raising the virulence, as will be seen from an inspection of the accompanying table, which is compiled from his paper.

Table I.

| Animal. | Inoculation. | Dose. | Mode of infection. | Result. |
|---------------|--|---------------------|--------------------|--|
| 1. Rabbit.... | Large quantity of cocci from agar plate suspended in 4 c.c. of a 4-day broth culture | 0·5 c.c. | Intracerebrally | Died 4th day. |
| 2. Guinea-pig | — | 0·3 „ | „ | Ill from 3rd to 10th day; recovered. Killed at 4 months. |
| 4. Guinea-pig | 5-day agar culture from Rabbit 1 | 1·5 loop (3 mgrms.) | „ | Died 6th day. |
| 5. Guinea-pig | Agar culture from Guinea-pig 4 | 1 loop (2 mgrms.) | „ | Died 4th day. |

The rise in virulence for the guinea-pig resulting from one passage through the rabbit is remarkable; but it will be seen that the additional passage through Guinea-pig 4 did not cause any appreciable effect—the fact that a slightly smaller dose was fatal to Guinea-pig 5 might be readily explained on the ground of that animal's greater susceptibility, *e.g.*, a younger or a smaller animal—a contention which is supported by the observations that in subsequent inoculations 2 and 1 loop (*i.e.*, 4 and 2 milligrammes) were fatal respectively in four and six days.

This feeble virulence for the guinea-pig combined with the tardy growth of *M. melitensis* upon artificial media renders it a matter of some difficulty to estimate the amount, or indeed the presence of protective bodies in the serum of animals treated with cultivations of the organism—so troublesome a process in fact, that after attempting, during the early part of last year, to prepare an anti-serum from the goat, I suspended my experiments in favour of an inquiry into the possibility of exalting the virulence of the micrococcus.

In view of the results of Durham's observations, I decided to limit my endeavour to exalt virulence for the guinea-pig to the intra-cerebral method of inoculation, and to employ, at first, one strain only of the *M. melitensis* in my experiments. The result was distinctly encouraging, for after rather more than a score of passages a very considerable access of virulence was obtained.

Before giving the details of these passages, a few points concerning the organism used and the methods followed may be of interest.

Organism Employed.—*M. melitensis* "No. 5."

Origin.—Isolated from the pus during life, and subsequently from the spleen (*post-mortem*) of a fatal case of subdiaphragmatic abscess* occurring in Guy's Hospital during October, 1903.

Morphology and Cultural Characters quite typical.

Initial Virulence for the Guinea-pig.—When first isolated, three entire four-day agar cultivations (*vide infra*) emulsified in 0.4 c.c. sterile saline solution, injected intracerebrally, were required to cause death—the fatal termination ensuing about 25 days after inoculation.

Medium Used for Growth of Virulence Cultures.

Previous experiments of my own, no less than the experience of other observers, show that fluid media are quite unsuited for the growth of, what I may term "Virulence Cultures." In the first place, growth in, for example, nutrient broth is extremely slow, and does not reach its maximum until the 6th to 8th day: then, too, the cranial capacity of the guinea-pig is small, and if the dose of culture exceeds or even amounts to 0.5 c.c., either some of the inoculated fluid at once escapes, or severe pressure symptoms supervene, and the animal dies within a few minutes.

A cultivation upon a solid medium, on the other hand, affords the opportunity of concentrating a large amount of the infective material in a bulk sufficiently small to ensure retention of the entire dose within the cranial cavity, without causing the exhibition of pressure symptoms, by scraping off large numbers of cocci and emulsifying in minute quantities of sterile saline solution.

Some observations upon the cultural characters of *M. melitensis* made during last summer by the writer in conjunction with Surgeon Duncan, R.N., showed that upon ordinary nutrient agar (prepared and standardised to +10, according to the method and scale I have described elsewhere)† *M. melitensis* in subcultivations would develop colonies

* Eyre, "A Case of Subdiaphragmatic and Hepatic Abscess consecutive to Mediterranean Fever," 'Guy's Hospital Reports,' 59, 1905, pp. 207—216.

† Eyre, "Standardisation of Nutrient Media," 'Brit. Med. Journ.,' 2, 1900, p. 921, and 2, 1901, p. 788.

visible to the naked eye in from 24 to 30 hours at 37° C., and would attain the maximum development in from 72 to 96 hours.*

I therefore decided to employ this medium in the form of "slant" tube cultures, filling the medium in quantities of 10 c.c. into tubes specially selected to secure uniformity of size, and always slanting the medium at about the same angle in order to obtain approximately equivalent areas for growth in each culture.

Preparation of Inoculum.

Tubes were inoculated from the spleen of one guinea-pig which had succumbed to intracerebral infection for inoculation into the brain of the next animal of the series, and after 24 hours' incubation at 37° C. were examined with the naked eye, and microscopically by means of smear preparations. As a rule at this age no definite colonies could be distinguished with the naked eye, although the inoculated surface of the medium presented a ground-glass appearance, and film preparations always yielded abundant evidence of growth.

After the preliminary examination, a sterile platinum loop was introduced into the tube, moistened in the water of condensation, and then gently rubbed all over the slanted surface of the medium. As the result of this manoeuvre, by the third or fourth day the medium was covered with a luxuriant growth, affording ample material for inoculation.

Method of Measuring the Dose of Inoculum.

The terms "entire culture," "half a culture," etc., as applied to dosage have little to recommend them on the score of exactitude, therefore the more accurate method of measurement by "loops" evolved by the late Dr. Washbourn and myself for the estimation of the virulence of the pneumococcus was utilised in the experiments. Briefly, this method consists in using a platinum loop accurately calibrated by weighing experiments; filling it carefully with the culture, and then emulsifying its contents in a definite measured quantity of broth, and using for the inoculation portions of this emulsion, representing fractions of the original loop. The special loop I use is the one originally made for pneumococcus work; its holding capacity had already been estimated to equal 0.5 milligramme of a 24-hour-old blood agar cultivation of pneumococcus; and when re-calibrated for 72 to 96-hour-old agar cultures of *M. melitensis* it was determined to have an identical capacity.

* At the same time I am not prepared to state absolutely that +10 is the optimum reaction of the medium to be employed for this purpose, until some experiments that are being carried out now are completed. Still this reaction is undoubtedly close to the optimum, so that in the absence of more definite knowledge I did not feel inclined to deviate from it.

Further, by plating out various fractions of a loopful of 72 to 96-hour-old agar cultivation and enumerating the colonies that subsequently developed, an approximate estimation was obtained of the number of cocci per loopful—that is to say, contained in 0.5 milligramme cultivation.

The average determined from a large series of experiments worked out to 1,250,000,000 cocci per loopful.

Now as in some of the preliminary inoculations more than one “slant” tube culture was required to produce a fatal infection, several observations were made to determine the average number of loopfuls per cultivation. After many trials this was found to be 25; and all the doses in the detailed table of inoculations are calculated as “loops,” and so recorded—although in the first few inoculations the figures can only be regarded as approximately correct, for these doses were not measured accurately, on account of their size.

Method of Inoculation.

As the intercranial method of inoculation is not amongst those most commonly practised, some details of the *technique* I adopted may be of interest.

The guinea-pig is first fully anæsthetised by means of a mixture of alcohol, chloroform, and ether, in the proportion of 1 : 2 : 6 (A_1 , C_2 , E_6), administered on a piece of absorbent cotton-wool placed either in the corner of a folded towel, or in the bottom of a small conical glass beaker.

The animal is then fastened down to the operating table, or firmly held by an assistant, and the hair of the scalp moistened with a solution of soft soap in 2-per-cent. lysol, which, with the help of hot water and cotton-wool, is worked up into a lather. The entire scalp, from the occipital protuberance to the root of the snout, is shaved, and finally washed with warm lysol solution.

A median incision commencing over the occiput and running forwards for about 2 cm., is made through the skin and subcutaneous cellular tissue, and retractors, secured by the assistant, used to hold open the wound. The periosteum is next divided along the entire line of the skin incision, then raised with a blunt dissector and also secured by the retractors.

A small nasal trephine (Curtis's), having a tooth-cutting circle of 6 mm. diameter,* is attached to a dental engine, and a small disc of bone removed from the left parietal bone; this trephine hole is cut well to one side of the median line to avoid injuring the superior

* This instrument has been adapted for me by Messrs. Down Bros. by the addition of an adjustable collar guard, secured by a screw, to prevent laceration of the dura mater or brain substance.

longitudinal sinus, a mishap which gives rise to troublesome hæmorrhage.

A hypodermic syringe provided with a fine needle is used to inject the measured dose of cocci, and some little manipulation is found to be necessary to ensure that the animal receives the entire dose. The injection may be made into any portion of the brain substance, or into the subdural space. Usually I inject into the left cerebral hemisphere, rarely into the frontal region. I avoid entering the cerebellum solely because muscular tremors and twitchings of the entire body are thereby induced which last for some minutes and interfere with the suturing of the skin wound.

The disc of bone is replaced or not, according to circumstances. If the injection appears to have caused any appreciable rise in the intracranial pressure, as indicated by protrusion of brain matter and meninges into the trephine circle, I do not replace the bone; otherwise I do. The periosteum is now readjusted as nearly as possible to its original position, and the skin incision closed by means of a continuous suture of either linen or silk, then sealed with flexile collodion. A dressing of sterile absorbent cotton-wool is fixed over the wound with more collodion, and the animal allowed to come round from the anæsthetic. Although the description is lengthy, the operation occupies but little time; given one assistant to attend to animal and the anæsthetic, 10 minutes will suffice from the commencement of the anæsthetisation to the return of the guinea-pig to its cage.

Passages to Exalt Virulence.

For the sake of brevity and clearness, I have tabulated the details of such of my inoculation experiments* as are pertinent to the present inquiry (*vide* Table II), and in this connection I must distinctly point out that my object was in no sense to determine the *minimal* fatal dose of that particular strain of *M. melitensis* I employed, for I take it the minimal fatal dose is the smallest dose which will cause a fatal specific infection after the lapse of no matter how lengthy a period.

Such an inquiry would have required the expenditure of more time than is at the disposal of any one man, for Durham has already shown, and I can fully confirm his observations, that an experimental animal may die of *M. melitensis* infection at a period as far distant as three months from the date of inoculation.

My intention was rather to so raise the virulence of the coccus for the guinea-pig that a comparatively small, and accurately measurable dose, should consistently cause death within a definite period of seven days; and in tabulating my results I have been guided by this principle, and have restricted myself as far as possible to the inclusion of

* Eyre, 'The Preparation of Nutrose Agar,' 'Trans. Path. Soc.,' 55, 1904, p. 91.

those animals in any given series that succumbed within seven days to the smallest dose. For instance, Guinea-pig 12 was in fact labelled (B) of a series of three inoculations performed on the same day with different doses of the same culture. (A) received 25 loops, and died in 21 hours; (C) received 0.1 loop, and died in 12 days; therefore (B), having received the smallest dose (1 loop) that was fatal within the prescribed period, alone appears in the table.

From the details shown it will be seen that after 21 passages through guinea-pigs the virulence of a particular strain of *M. melitensis* originally so feeble that 75 loops (or 37.5 milligrammes) of culture required 25 days to kill a 380-gramme guinea-pig, has been so exalted that two loops (or 1 milligramme) of culture is sufficient to kill a 590-gramme pig in about 24 hours, whilst 0.5 loop (or 0.25 milligramme) will kill a 350-milligramme pig within five days.

Course of the Infection.

The course of the infection of the guinea-pig by *M. melitensis* may be conveniently considered under two separate headings: (1) Acute, and (2) Chronic, according to whether death is caused in a few hours or days, or is delayed for from one week to two or three months.

Acute Infection.—An animal dying within a few days of intracerebral inoculation with a moderate dose of a highly virulent cultivation or a large dose of a less virulent one, supplies the type for this form of *M. melitensis* infection.

A short incubation period varying in duration from 12 to 24 hours follows the inoculation, and during this time the animal appears to be in normal health and eats well, although the progressive loss of weight which is the marked characteristic of the infection begins within a few hours of inoculation. A stage of irritation follows the incubation period, and lasts for about 24 hours; it is marked by convulsions, at first localised and produced in response to direct stimuli; afterwards becoming generalised, tonic in character and occurring at frequent and irregular intervals; progressive muscular weakness is a marked feature of this stage, throughout which the animal is obviously ill and stupid, and refuses food. The stage of irritation passes gradually into one of coma, with paresis or paralysis, affecting first the hind legs, afterwards involving the fore limbs also. Handling or even touching will at first rouse the animal and provoke general convulsions; later, the guinea-pig falls on its side, becomes insensible, and, in fact, appears moribund. In this condition, however, the animal may remain for 24 or even 36 hours, and during the latter part of this period no rectal temperature can be recorded by the ordinary clinical thermometer, for 32° C. is hardly ever exceeded. Death is sometimes preceded by convulsions, but usually no such warning is given. To give a concrete

illustration of the train of symptoms and *post-mortem* findings in these acute infections I cannot do better than cite in full the clinical history of and autopsy on guinea-pig 19 (*vide* Table II), which is quite typical. Incidentally, I may mention that this case would serve equally well to illustrate the course of infection in the rabbit.

Guinea-pig 19. Sex ♂. Weight 450 grammes. Temp. 38° C.

| | | |
|---------|------------|---|
| 11.2.05 | 4 P.M. | A.C.E. was administered, and a 6 mm. trephine circle was cut from left parietal bone. Four (4) loops of 3-day old agar cultivation of <i>M. melitensis</i> from spleen of Guinea-pig 18, emulsified in 0.2 c.c. sterile saline solution, injected into substance of left cerebral hemisphere. Disc of bone replaced, also periosteum, skin incision sutured, and wound dressed with collodion and cotton-wool. |
| | 12 P.M. | Appears quite well. Has eaten well since inoculation. |
| 12.2.05 | 9 A.M. | Is huddled up in one corner of cage; is not eating; hair dull and standing on end; is obviously ill. Has lost 60 grammes in weight. |
| 13.2.05 | „ | Condition apparently unchanged. Has lost a further 60 grammes in weight. |
| | 10.15 A.M. | Is now grinding teeth, moves slowly, and, if turned on back, rights itself very slowly. |
| | 10.30 A.M. | Generalised spasms result if touched; convulsive movements occur from time to time even in the absence of obvious stimuli. |
| | 1 P.M. | Much worse—marked paresis of hind quarters. |
| | 2 P.M. | Convulsive “circus” movements occur from time to time—the animal dragging itself round “clockwise” by means of its fore paws. |
| | 9 P.M. | Quiet in corner of cage; breathing laboured. |
| 14.2.05 | 9 A.M. | Apparently unconscious, lying on side; if placed on legs is unable to stand, and falls down after slight feeble convulsive movements, once more becomes still. Breathing shallow and slow. |
| | 11.30 A.M. | Condition unaltered. |
| | 11.45 A.M. | Still in same position as when last looked at. Is now dead. |

Post-mortem Examination.

Scalp Incision.—Scalp wound healthy in appearance, lips of incision healing by primary union; no signs of pus visible in wound; no stitch abscesses.

Subcutaneous tissue occupied by cedematous and jelly-like exudation marked here and there with small hæmorrhages.

Bone.—The disc of bone is firmly fixed *in situ* by serous exudation, in which the periosteum is also involved. On raising the disc of bone no protusion of meninges, etc., occurs.

Cranial Cavity.—(This is most conveniently reached without damage

to the structures beneath by reflecting skin and periosteum from over the entire cranial vault; then, with a red-hot iron, thoroughly searing the bone, which when seared and dry can easily be broken up with a pair of forceps, and removing it piece-meal, starting from the trephine circle.)

General congestion and injection of the vessels of the dura mater, the site of inoculation being marked out by an area of bloody lymph, roughly corresponding in size and shape to the circle of bone removed from the calvarium. On removing the meninges a thick layer of yellowish lymph is seen adhering to the surface of the convolutions in the left parieto-occipital region (this microscopically consists of a dense mass of large mononuclear leucocytes, permeated throughout by masses of cocci); cerebral vessels greatly engorged and numerous petichial hæmorrhages visible on the surface of the brain.

The cerebro-spinal fluid is more or less increased in amount and contains numerous micrococci, free and also included in cells.

On section there are numerous small hæmorrhages scattered throughout the brain substance, whilst the *velum interpositum* is so congested as to resemble a clot of blood. Elsewhere the brain appears unaffected to the naked eye. Agar tubes inoculated with brain substance from any portion of cerebrum or cerebellum or from cerebro-spinal fluid, either cerebral or spinal, yield a copious and pure growth of *M. melitensis* within 36 hours.

Thoracic Cavity.—Slight enlargement of anterior mediastinal and of bronchial glands. Small quantity of clear serous effusion in the pleural cavity. Cultivations from this fluid remain sterile. Few hæmorrhages on the surface of lungs. Pericardium distended with clear serous fluid, also sterile. Blood removed from right side of heart plated in agar yields, on an average, some 35 colonies per cubic millimetre.

Peritoneal Cavity.—Excess of clear blood-stained fluid in peritoneal cavity, sterile on cultivation. Gall-bladder distended with clear bile. Liver, spleen, kidneys dark and engorged with blood, the spleen being distinctly enlarged.

Cultivations from liver and spleen give good growth of *M. melitensis* within 48 hours. Kidney pulp yields only a few scattered colonies of *M. melitensis*.

Omentum injected; a few enlarged mesenteric glands noted.

Bladder distended with turbid urine. Cultivations prepared from the centrifugalised deposit of the few cubic centimetres of urine contained in the bladder remain sterile.

Cultivations from the bone marrow from practically all the long bones yield a more luxuriant growth of *M. melitensis* than from other organs, with perhaps the exception of the spleen and brain.

Chronic Infection.—As in the present instance we are not particularly concerned with chronic infection, I shall dismiss this subject very briefly.

After intracerebral inoculation with a very minute dose of a highly virulent culture or a fair sized dose of a less pathogenic one the infection pursues an extremely chronic course, and beyond progressive emaciation and profound anæmia presents no very marked or characteristic symptoms. The early symptoms resemble those of the more acute infection above described but are much less severe in character. For instance the incubation period is usually prolonged to two or three days and is followed by a period, extending over from three to six days, during which the animal is distinctly ill and refuses its food, remains huddled up in one corner of its cage, loses weight rapidly and becomes extremely weak. Convulsions of a mild type can usually be provoked at the beginning of this stage by handling the animal or by turning it on to its back.

The animal then gradually recovers, eats well—even ravenously—and although the emaciation may be arrested for a while, the original weight is never entirely recovered. After an interval extending over weeks or even months, during which, except for emaciation, the animal appears in perfect health, death suddenly takes place.

More rarely the animal is obviously ill for two or three days before death, refuses food and becomes comatose just before the end.

Post-mortem Appearances.

Seat of Inoculation.—The site of the skin incision is occupied by a firm linear scar usually adherent to the periosteum and bone beneath, the disc of bone, if it has been replaced, has usually united completely.

Cranial Cavity.—Slight injection of meningeal and cerebral vessels usually present—brain substance appears normal.

Cultures from brain substance and cerebro-spinal fluid yield only a scanty growth of *M. melitensis* or remain sterile.

Thoracic Cavity.—Lungs usually anæmic, otherwise normal; cultures from heart blood remain sterile.

Peritoneal Cavity.—Peritoneum and intestines blanched and anæmic; no subperitoneal, omental or mesenteric fat visible; otherwise viscera normal. Cultivations from liver remain sterile; those from spleen and bone marrow may or may not yield a scanty growth; on the other hand those established from the centrifugalised deposit of the turbid urine or even from the urine itself give rise to fairly good growth.

Mode of Exit of M. melitensis from the Body.

A matter of the highest practical importance, but one upon which I have not yet touched, deals with the route or routes by which *M. melitensis* leaves the animal body. So far as concerns what we have termed “chronic” infections, *M. melitensis* can be readily isolated from catheter specimens of the urine throughout the course of the infection and also from urine taken directly from the bladder *post-*

mortem, even when intervals measured by months have elapsed since the inoculation, and no matter what the path of the original infection of the animal has been—intraperitoneal, subcutaneous, or intracranial. This was shown first by Durham, and his results, so far as concerns the guinea-pig and rabbit, have subsequently been as fully confirmed by the observations of other workers as by my own experiments; Horrocks and Shaw have recorded analogous results, too, from observations upon the human subject.

When, however, we consider the results of observations upon the *acute* infections, we find that *M. melitensis* is not consistently present in the urine. In about 50 per cent. of the animals dying within five days of inoculation, I have failed to detect the Micrococcus in the urine, although I have employed the entire contents of the bladder for the insemination of culture tubes and plates.

During the experiments detailed above, which had for their primary object the exaltation of virulence, I systematically examined many of the organs of the infected animals by cultural tests, using a special circular loop of 1.5 mm. diameter, and carefully compared the amount of the resulting growths.

My observations soon convinced me that the most copious growth per loopful of material was obtained from the brain substance, next in order and all about equal came the liver, spleen, and long bone marrow. Finding such large numbers of cocci present in the liver tissue, I naturally turned my attention to the contents of the gall bladder. In my first observations I seared the surface of the gall bladder with a red hot iron at some convenient spot, punctured the wall with a Pasteur pipette, and aspirated some of the bile in order to prepare my cultivations.

My results were unsatisfactory and inconsistent—sometimes a good growth of *M. melitensis* was obtained; at others the cultures remained sterile. My positive results, I noticed, were obtained when the cultures were made immediately after death; usually, when the *post-mortem* examination was not performed until some hours after death, a negative result was recorded.

It was next observed that if the cadaver was allowed to remain undisturbed, sedimentation occurred in the bile, and the cocci became collected into large flocculent masses deposited near the mouth of the common bile duct, so that if cultivations were made from the supernatant bile no growth resulted, although good growth could be obtained from the before-mentioned flocculent masses when these were taken up by the pipette in aspirating the last portion of bile.

Following the course of the bile, in my subsequent *post-mortem* examinations I prepared plate cultivations (using the modified Drigalaki and Conradi "nutrose" medium that I have already described in connection with some dysentery investigations) from

Table II.—Intracerebral Passages to Exalt Virulence.

| Guinea-pig. No. in series. | Sex. | Weight in grammes. | Dose of inoculum. | | Bulk of inoculum. | Duration of infection. | Weight at death, in grammes. | Observed loss of weight. | |
|-------------------------------|------|--------------------------|-------------------|---------------------|----------------------|---------------------------|------------------------------------|--------------------------|-------------------------------|
| | | | In looppuls. | In milligrammes. | | | | Total in grammes. | Percentage of body weight. |
| 1 | ♂ | 380 | 75 | 37.5 | 0.4 | 25 days | 160 | 220 | 78.5 |
| 2 | ♀ | 420 | 25 | 12.5 | 0.3 | 15 " | 150 | 270 | 64.2 |
| 3 | ♂ | 460 | 25 | 12.5 | 0.2 | 7 " | 250 | 210 | 45.6 |
| 4 | ♂ | 700 | 25 | 12.5 | 0.3 | 9 " | 350 | 350 | 50 |
| 5 | ♀ | 750 | 25 | 12.5 | 0.3 | 2½ " | 630 | 130 | 17.3 |
| 6 | ♀ | 480 | 25 | 12.5 | 0.2 | 2½ " | | | |
| 7 | ♀ | 560 | 25 | 12.5 | 0.2 | 2 " | | | |
| 8 | ♀ | 560 | 12 | 6 | 0.3 | 2 " | 450 | 110 | 19.6 |
| 9 | ♂ | 890 | 12 | 6 | 0.3 | 3 " | 710 | 180 | 20.2 |
| 10 | ♂ | 570 | 12 | 6 | 0.2 | 3½ " | 460 | 110 | 19.3 |
| 11 | ♂ | 870 | 6 | 3 | 0.2 | 2 " | 270 | 100 | 27 |
| 12 | ♂ | 310 | 1 | 0.5 | 0.2 | 4½ " | 200 | 110 | 35.4 |
| 13 | ♂ | 240 | 12 | 6 | 0.2 | 2½ " | 210 | 130 | 54.1 |
| 14 | ♂ | 240 | 15 | 7.5 | 0.2 | 4 " | 210 | 130 | 54.1 |
| 15 | ♂ | 240 | 5 | 2.5 | 0.1 | 24 hours | 200 | 40 | 16.6 |
| 16 | ♀ | 240 | 8 | 4 | 0.2 | 2 days | 165 | 75 | 31.2 |
| 17 | ♂ | 180 | 6 | 3 | 0.2 | 2 " | 150 | 30 | 16.6 |
| 18 | ♂ | 350 | 6 | 3 | 0.2 | 2½ " | 320 | 30 | 8.5 |
| 19 | ♂ | 450 | 4 | 2 | 0.2 | 3 " | 280 | 170 | 37.7 |
| 20 | ♂ | 250 | * | * | = | 21 hours | 210 | 40 | 16 |
| 21 | ♀ | 380 | 2 | 1 | 0.1 | 3½ days | 270 | 110 | 28.9 |
| 22 | ♀ | 590 | 2 | 1 | 0.3 | 24 hours | 460 | 130 | 22 |
| 23 | ♂ | 350 | 0.5 | 0.25 | 0.1 | 5 days | 230 | 120 | 34.2 |
| 24 | ♂ | 480 | 0.5 | 0.25 | 0.1 | 2 " | 360 | 100 | 21.7 |
| 25 | ♀ | 490 | 0.5 | 0.25 | 0.1 | 3 " | 330 | 110 | 25.5 |

* Owing to error, no note was made of the size of the dose.

Table III.—Post-mortem findings.

| Guinea-pig. No. in Series. | Micrococcus melitensis recovered in culture from— | | | | | | | | | | | | | | | | |
|-------------------------------|---|--------------|------------------------------------|---------|--------|-------|----------------------|---------|--------|----------------------|-----------|----------|--------|--------|---------|--------|-----------------------|
| | Brain sub- stance. | Heart blood. | Serous effu- sion in thorax. | Spleen. | Liver. | Bile. | Peritoneal fluid. | Kidney. | Urine. | Long bone marrow. | Duodenum. | Jejunum. | Ilium. | Cæcum. | Rectum. | Fæces. | Intestinal mucous. |
| 1 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 4 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 5 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 6 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 7 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 8 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 9 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 10 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 11 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 12 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 13 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 14 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 15 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 16 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 17 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 18 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 19 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 20 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 21 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 22 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 23 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 24 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |
| 25 | + | + | | + | + | + | | + | + | + | + | + | + | + | + | + | + |

+ = growth of *M. melitensis*. ± = scanty growth of *M. melitensis*. - = no growth of *M. melitensis*. 0 = not examined.

scrapings from the walls of the bile duct, and from the mucous membrane lining of the duodenum, and had no difficulty in demonstrating the presence of fair numbers of the Micrococci in both these situations. Proceeding onwards along the intestinal canal, positive results were obtained from the upper portions of the jejunum, but owing to the enormous numbers of purely intestinal bacteria which rapidly overgrew the plates, I was at first unable to demonstrate *M. melitensis* in the lower portion of the jejunum, the ileum, cæcum or rectum.

With the death, however, of Guinea-pig 20 (*vide* Table II) within 24 hours of inoculation, a positive result was obtained from each of these situations, although as the rectum was empty no observations could be carried out with regard to the characteristic faecal masses.

Guinea-pig 21 (*vide* Table II) was deposited in a sterilised glass dish whilst in a moribund condition, so that when the contents of the rectum were expelled at death an hour later, it became a simple matter to prepare plate cultivations from intestinal mucus and faeces separately.

From the mucus a fair number of Micrococci was isolated; the faecal masses gave considerably more trouble on account of the numerous members of the coli and streptococcus groups that were present, but eventually several colonies were isolated and completely identified with *M. melitensis*.

As will be seen from the details of the *post-mortem* examinations (Table III), these results were confirmed more than once.

From the results of these experiments, therefore, the assumption is justified that *M. melitensis* leaves the body of experimental animals by way of the intestinal tract, and possibly by way of the urinary tract also, when the infection is of the acute type. That the coccus leaves the body by way of the urinary tract *alone* when the infection is of a more chronic character appears probable also, for not only have I not succeeded in isolating it from the alimentary canal or faeces of such animals, but cultivations from liver and bile have always yielded negative results.

Conclusions.

1. By a series of intracerebral inoculations, comprising rapid passages from guinea-pig to guinea-pig, the virulence of *M. melitensis* can be exalted to a high pitch for this particular animal.

2. The virulence finally obtained in the present series is such that a small and accurately measurable dose of cultivation corresponding to about 0.25 milligramme in weight, or to rather more than 6,000,000 cocci, will consistently cause death in about five days.

3. Experimental observations show that in these acute infections *M. melitensis* leaves the body of the inoculated animal by way of the alimentary canal, in the intestinal mucus and the faeces, as well as occasionally by way of the urinary tract (in the urine).

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REPORTS OF THE COMMISSION FOR THE INVESTIGATION OF MEDITERRANEAN FEVER.

Part I.

Containing Reports by

Major HORROCKS, R.A.M.C., Staff-Surgeons GILMOUR and
SHAW, R.N., and Dr. ZAMMIT.

26.854

REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

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OF

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INTRODUCTION.

In the Introduction to Part I, the history of this investigation into the causation and prevention of Mediterranean Fever was given from its commencement until the end of the summer of 1904.

Major W. H. Horrocks, R.A.M.C., and Dr. R. W. Johnstone, Local Government Board, left Malta at the end of September and arrived in England on October 8, 1904.

The results of the work of the Commission during the summer of 1904 are published in Parts I and II.

At a meeting of the Sub-Committee, held on November 17, 1904, it was decided that Staff-Surgeon E. A. Shaw, R.N., Dr. T. Zammit, Board of Health, Malta, and Captain J. Crawford Kennedy, R.A.M.C. should continue the work during the coming winter, and that Major Horrocks and Dr. Johnstone be asked to return to Malta at the beginning of the following fever season. As Captain Kennedy had been struck off all military duty and was devoting his whole time to the work of the investigation, he was made a member of the Commission.

Major Horrocks returned to Malta about the end of May, 1905, but Dr. Johnstone was unable to take up the work again this summer. Lieut.-Colonel A. M. Davies, R.A.M.C., was therefore appointed a member of the Commission in his place and arrived in Malta on May 28, 1905.

Colonel Bruce, C.B., F.R.S., R.A.M.C., the Chairman of the Sub-Committee, left England on May 19, 1905, and proceeded to Malta, where he met the members of the Commission. Staff-Surgeon Shaw and Captain Kennedy handed to him the papers which form part of the present volume. Dr. Zammit informed him that on account of the pressure of other duties he had been able to do but little work for the Commission, but that he would now be able to devote his whole time to it. He communicated some notes on the feeding of goats with *Micrococcus melitensis*, which seemed to show that the goat is to some extent susceptible to Mediterranean Fever. The following experiments were made by Dr. Zammit:—

Experiment 1.—White Goat.

To note the effect of feeding goats on material containing *Micrococcus melitensis*.

1904—

Sept. 15. Examined blood for agglutination. Negative.

„ 18. Fed this goat, adding the contents of a culture of *M. melitensis* on agar to its food.

Dec. 3. Blood has reacted in dilutions of 1 in 20 to 1 in 100, but the temperature curve shows no rise. Fed again in the same way.

„ 23. Blood reacts 1 in 300.

1905—

Apr. 29. Blood reacts 1 in 100. Goat still alive.

Experiment 2.—Red Goat.

1904—

Dec. 3. No blood reaction. Fed one tube agar.

„ 5. Fed again.

„ 15. No blood reaction.

„ 23. Blood reacts 1 in 20 ; 1 in 50 after half-an-hour.

1905—

Apr. 29. Blood reacts 1 in 50.

Dr. Zammit informed the Chairman that he considered goats to be susceptible to Mediterranean Fever, and that the disease is spread to human beings by goats. A temporary laboratory was set up in the Lazaretto buildings, Fort Manoel, to continue the investigation of the disease in goats and also the transference of the disease by mosquitoes.

Colonel Bruce returned to England on June 12, 1905.

On June 23, 1905, Major Horrocks wrote that he had discovered the *M. melitensis* in the milk of an apparently healthy goat, and on the 26th he further wrote that he had already found the *M. melitensis* in the milk of five goats taken from two different herds, and that Dr. Zammit had found it in the blood of one of these goats. Horrocks also said that the milk of the goat fed by Dr. Zammit last September was still crammed with *M. melitensis*. It would therefore appear that the Commission are on the eve of an important and may be far-reaching discovery.

On July 18, 1905, the Chairman received preliminary notes from Major Horrocks, Captain Kennedy, and Dr. Zammit, on the propagation of Malta Fever by means of goats. These are added to the present volume.

These notes show (1) that one or more apparently healthy goats in every herd are excreting *M. melitensis* in their milk and urine ; (2) that about 50 per cent. of the goats in Malta react to Mediterranean Fever when examined by the serum agglutination test.

It may be objected that no exact proof exists that the drinking of milk containing *M. melitensis* will give rise to the disease in man. When we take, however, into consideration the results of the feeding and inoculation experiments on monkeys, it may be assumed with safety that the disease is propagated in this way, and that no time should be lost in removing such a grave and insidious danger to the public health.

I. ON A QUANTITATIVE BACTERIOLOGICAL EXAMINATION OF THE BLOOD OF 103 MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

Blood.

In my September Report I gave the results of the examination of the peripheral blood of 51 Malta Fever patients for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*). I now give briefly the results of a further series of 52 such cases similarly examined, making a total of 103 cases examined. If any points seem inadequately explained a reference to the first Report will elucidate matters.

Method.—Bend of elbow prepared as for a surgical operation, carbolic acid being the disinfectant used, a pad of lint soaked in 1 in 20 of this being kept on site of intended puncture till the latter was made. Five c.c. of blood drawn off from median basilic vein in graduated sterile serum syringe; 3 c.c. of this placed in a flask containing 60 c.c. of peptone broth, 1 c.c. into a tube containing 19 c.c. of peptone broth and 1 c.c. into a second tube also containing 19 c.c. of broth, and all these well shaken. The flask containing 3 c.c. of blood and one of the tubes containing 1 c.c. of blood were incubated intact. The second tube containing a mixture of 19 c.c. of broth and 1 c.c. of blood was treated as follows: half of its contents were removed with a 10 c.c. sterile pipette and it was then put aside, now containing $\frac{1}{2}$ c.c. of blood and $9\frac{1}{2}$ c.c. of broth, to be incubated; the contents of the 10 c.c. pipette were then added to a 10 c.c. broth-tube, which was well shaken, and now contained 20 c.c. of fluid, $\frac{1}{2}$ c.c. of which was blood; 10 c.c. of this mixture was removed with the 10 c.c. pipette, leaving it containing $\frac{1}{4}$ c.c. of blood, and it was put aside to be incubated; the contents of the pipette were added to another 10 c.c. broth-tube, which in its turn was left with $\frac{1}{8}$ c.c. of blood, and so on, halving the quantity of blood each time till the following series was obtained: flask containing 3 c.c. of blood, tubes containing 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood duly numbered and dated, these were then placed in the incubator at 37° , being taken out daily and well shaken to facilitate distribution of the possible *M. melitensis* throughout the medium. At the end of a week glucose-litmus-agar slopes were inoculated by means of a loop from

the 3 c.c. flask, 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ c.c. tubes, these duly labelled and all returned to the incubator. At the expiration of another two days these slopes were examined and those which exhibited growth were put aside to be put through the usual tests for *M. melitensis* (see September Report), and the others with the original broth-tubes returned to the incubator. At the end of a second period of two days the agar slopes were again examined, those showing growth being dealt with as before, and now, it being 11 days since commencement of incubation, those slopes which showed no growth were reinoculated plentifully from the corresponding broth-tubes, and the remainder of the series, the $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$, and $\frac{1}{512}$ c.c. blood broth-tubes, were sub-cultured by means of a large platinum loop on to agar slopes, and all returned to the incubator for a further period of three days, after which all slopes were examined for growth, results recorded, and the examination of that particular specimen of blood terminated.

The foregoing was the general method adopted. It was only slightly departed from in the last 25 cases; in which in order to determine the earliest date at which in these examinations *M. melitensis* made its appearance in the broth, daily sub-cultures were made from the flask containing the 3 c.c. of blood starting from the first day of incubation. As this was never found to be later than the eighth day, and as it will be observed from the foregoing that a total of eleven days' incubation in broth, the same period as observed in the first series of 51 cases, was completed before a blood broth-tube was abandoned as unfruitful, there is obviously an ample margin of safety, and I do not consider that there was any possibility of any fruitful blood broth-tube having been overlooked.

The following table gives the result in a very compressed form; it seems to me unnecessary to write out each blood examination separately. In the remarks which follow the tables, such cases as call for it are discussed in greater detail individually. As all the cases in this series were English and male, nine patients belonging to the navy, and 43 to the army, no column descriptive of nationality and sex has been necessary. In the column "stage of fever," the word "wave" refers to waves of raised temperature. In the column giving patients' temperature for a few days prior to bleeding, the last temperatures are those for the morning and evening of the day blood was drawn, those preceding it being arranged in regular chronological order; the intention being by comparing these, in the cases yielding *M. melitensis*, to ascertain if any relation existed between course of temperature and presence of *M. melitensis* in the blood; these temperatures are given in the form of a fraction, the numerator being the morning and the denominator the evening temperature, the one taken about 8 A.M. the other about 5 P.M. Day of disease, as before, has been calculated from the day the patient first began to feel ill, not from date of

admission into hospital. In each case the highest dilution of the patient's blood serum (determined from a portion of the blood taken for bacteriological examination), which gave a distinct agglutination reaction in a quarter of an hour under the $\frac{2}{3}$ -in. objective of the microscope, has been worked out and is given in the appropriate column as a dilution of 1 in ..., the unit being serum, the other numeral being so many equivalent bulks of "normal" physiological salt solution.

In the last column "recovery of and smallest quantity of blood yielding *M. melitensis*," "Nil" signifies that in this case the result of the examination was negative. The minimal quantity of blood yielding *M. melitensis* is given as in decimals of a cubic centimetre, a preference having been expressed for this mode of presenting it. To facilitate comparison with the first series in which this quantity was expressed as a fraction, I give the equivalent from $\frac{1}{8}$ onwards—

$$\begin{array}{lll} \frac{1}{8} \text{ c.c.} = 0.1250 \text{ c.c.} & \frac{1}{16} \text{ c.c.} = 0.0625 \text{ c.c.} & \frac{1}{32} \text{ c.c.} = 0.0312 \text{ c.c.} \\ \frac{1}{64} \text{ c.c.} = 0.0156 \text{ c.c.} & & \frac{1}{128} \text{ c.c.} = 0.0078 \text{ c.c.} \\ \frac{1}{256} \text{ c.c.} = 0.0039 \text{ c.c.} & & \frac{1}{512} \text{ c.c.} = 0.0019 \text{ c.c.} \end{array}$$

In the chronological table which comes immediately after the foregoing, fractions have had to be resorted to, because when, as in the 17th and 22nd days of the disease, the results of as many as six blood examinations had to be put down in one space, the use of decimals was found to result in an agglomeration of figures in which "definition" was greatly lacking. Here also the foregoing equivalents may be found useful.

| No. of case. | Age. | Stage of the fever. | Temperature of patient for few days preceding bleeding. | ° F. | Day of disease. | Time of bleeding and patient's temperature. | Maximum dilution of patient's blood giving agglutination. | Recovery of and smallest quantity of blood yielding <i>M. melitensis</i> . |
|--------------|------|---------------------------|---|-------|-----------------|---|---|--|
| 52 | 21 | { Nearing end of 1st wave | 99·4, 98·6, 98·4, 100, 98·4 | | 35th | 10.40 A.M., 98°·6 | 1 in 200 | c.c. 0·5 |
| 53 | 20 | { Middle of 1st wave | 101·6, 100, 98·6, 98·6, 98·4 | | 19th | 10.55 A.M., 99°·2 | 1 in 500 | 0·125 |
| 54 | 20 | { Middle of 1st wave | 98·6, 99·4, 102, 100·2, 100·8 | | 23rd | 11.5 A.M., 99°·4 | 1 in 2500 | Nil |
| 55 | 24 | { Still in 1st wave ... | 100·6, 101·4, 101, 99, 98·4 | | 33rd | 10.50 A.M., 99° | 1 in 200 | 0·25 |
| 56 | 35 | { In 2nd wave..... | 102·2, 102·6, 102, 101, 100·8 | | 116th | 11.0 A.M., 101° | 1 in 500 | Nil |
| 57 | 19 | { Middle of 2nd wave | 98·8, 98·6, 98·8, 98·8, 98·8 | | 96th | 11.10 A.M., 101°·2 | 1 in 5000 | 0·25 |
| 58 | 24 | { In 1st wave | 100, 100, 100·4, 99·8, 100·4 | | 17th | 10.50 A.M., 100°·4 | 1 in 100 | 0·0312 |
| 59 | 22 | { In 2nd wave..... | 98·2, 98·6, 99·2, 100·4, 100·8 | | 51st | 11.5 A.M., 100°·6 | 1 in 1000 | 2·00 |
| 60 | 29 | { In 2nd wave..... | 100·6, 101·6, 102·2, 102·8, 103 | | 70th | 11.0 A.M., 100°·4 | 1 in 500 | 0·0312 |
| 61 | 21 | { In 1st wave | 100·2, 100·4, 103·4, 101·4, 101 | | 9th | 11.10 A.M., 98°·6 | 1 in 2000 | Nil |
| 62 | 27 | { In 3rd wave | 102·8, 102·6, 102, 102·4, 102·4 | | 90th | 10.45 A.M., 98°·6 | 1 in 500 | 0·0078 |

| | | | | | | | |
|----|----|---|--|-------|--------------------|-----------|--------|
| 63 | 22 | In 1st wave | $\frac{98.4}{100}$, $\frac{98.2}{99.2}$, $\frac{98.4}{98.2}$, $\frac{98.6}{98.6}$, $\frac{98.2}{99}$ | 40th | 11.0 A.M., 98° 4 | 1 in 500 | 1.00 |
| 64 | 20 | { 1st wave not yet ended | $\frac{100}{97.8}$, $\frac{98.4}{102}$, $\frac{97.8}{100.8}$, $\frac{98.4}{101}$ | 108th | 11.10 A.M., 100° | 1 in 40 | Nil |
| 65 | 20 | In 1st wave | $\frac{101.6}{104.2}$, $\frac{103}{104}$, $\frac{100.2}{103.6}$, $\frac{98.2}{100.4}$, $\frac{98.4}{100.6}$ | 16th | 11.10 A.M., 100° 2 | 1 in 40 | Nil |
| 66 | 28 | Still in 1st wave ... | $\frac{101.4}{102.8}$, $\frac{100.4}{104}$, $\frac{101.4}{103.6}$, $\frac{101.6}{104.2}$, $\frac{103}{98.8}$ | 65th | 11.15 A.M., 102° | 1 in 500 | 0.0078 |
| 67 | 20 | In 1st wave | $\frac{98.4}{100.4}$, $\frac{99.4}{100.6}$, $\frac{98}{100}$, $\frac{98.8}{98}$, $\frac{100.2}{100.2}$ | 22nd | 11.10 A.M., 99° | 1 in 1000 | 0.125 |
| 68 | 28 | { Had 2 waves in 1st attack, then out of hospital 31 days, now in 1st wave of relapse | $\frac{98}{100.8}$, $\frac{98.6}{100.2}$, $\frac{99.4}{100}$, $\frac{100.6}{102}$, $\frac{98}{101.8}$ | 116th | 11.25 A.M., 98° 6 | 1 in 500 | Nil |
| 69 | 23 | In 1st wave | $\frac{100.6}{104}$, $\frac{101.2}{103.4}$, $\frac{93.6}{102}$, $\frac{98.2}{99.8}$, $\frac{98.2}{100}$ | 27th | 11.15 A.M., 99° | 1 in 20 | Nil |
| 70 | 41 | { In 1st wave (f a relapse | $\frac{98.6}{99}$, $\frac{98.2}{99.6}$, $\frac{98.6}{100}$, $\frac{98.6}{100.2}$ | 158th | 11.30 A.M., 99° | 1 in 40 | 0.25 |
| 71 | 20 | Still in 1st wave ... | $\frac{102}{104}$, $\frac{101.2}{103}$, $\frac{102}{103.4}$, $\frac{101.6}{103.6}$, $\frac{101.2}{102.6}$ | 56th | 12.15 A.M., 102° | 1 in 500 | 0.5 |
| 72 | 21 | In 1st wave | $\frac{98.4}{100.8}$, $\frac{99.4}{101.6}$, $\frac{100.2}{101}$, $\frac{99.2}{100.4}$, $\frac{99.2}{100.6}$ | 17th | 11.20 A.M., 99° 8 | 1 in 1500 | 0.0625 |
| 73 | 27 | In 1st wave | $\frac{99.6}{101.6}$, $\frac{102.4}{102.2}$, $\frac{102.8}{102.6}$, $\frac{102.6}{101.2}$ | 22nd | 11.30 A.M., 101° | 1 in 2000 | 0.5 |
| 74 | 25 | In 1st wave | $\frac{101}{103.2}$, $\frac{100.6}{103.6}$, $\frac{101.2}{103.4}$, $\frac{100.4}{103}$ | 18th | 11.45 A.M., 102° 1 | 1 in 2000 | 0.5 |
| 75 | 24 | In 3rd wave | $\frac{98.6}{100}$, $\frac{98.4}{100.2}$, $\frac{98.6}{99.8}$, $\frac{98.2}{103.3}$, $\frac{100}{100.6}$ | 149th | 4.45 P.M., 103° 3 | 1 in 100 | Nil |
| 76 | 20 | In 3rd wave | $\frac{98.4}{101.2}$, $\frac{98.8}{100}$, $\frac{98.4}{101.2}$, $\frac{99.2}{100.6}$, $\frac{98.5}{102.5}$ | 120th | 5.0 P.M., 102° 5 | 1 in 800 | 0.0625 |
| 77 | 21 | In 3rd wave | $\frac{98.4}{100.8}$, $\frac{98}{102.4}$, $\frac{98.6}{102}$, $\frac{98.6}{101}$, $\frac{98.4}{99}$ | 151st | 5.10 P.M., 99° | 1 in 300 | 1.00 |

| No. of case. | Age. | Stage of the fever. | Temperature of patient for few days preceding bleeding. | Day of disease. | Time of bleeding and patient's temperature. | Maximum dilution of patient's blood giving agglutination. | Recovery of and smallest quantity of blood yielding <i>M. melitensis</i> . |
|--------------|------|---|---|-----------------|---|---|--|
| 78 | 29 | In 1st wave | 98·4, 98·4, 98, 98·4, 99·4 102·2, 102·6, 101·6, 103·4, 103·6 | 35th | 10.45 A.M., 100° | 1 in 2600 | 1·00 |
| 79 | 20 | In 1st wave | 98·2, 97·8, 98·4, 98, 97·6 99·6, 101, 100·6, 100, 100·4 | 18th | 11.0 A.M., 98°·4 | 1 in 3000 | 0·125 |
| 80 | 22 | In 1st wave | 98·4, 98·4, 98, 97·4, 97·4 97·6, 97·4, 98, 98·4, 99 | 17th | 11.10 A.M., 98° | 1 in 2500 | 0·5 |
| 81 | 24 | In 1st wave | 100·2, 98·4, 99, 99, 99 101, 102·4, 99·2, 100, 100·2 | 12th | 4.40 P.M., 100°·2 | 1 in 500 | Nil |
| 82 | 27 | In 2nd wave | 99, 99·6, 98·4, 99·8, 99·2 102, 101, 101, 102·2, 101·6 | 67th | 4.55 P.M., 101°·6 | 1 in 1500 | 1·00 |
| 83 | 37 | In 1st wave | 100·6, 99·4, 100·4, 99, 97·8 102·4, 101·6, 100·6, 100·4, 100 | 18th | 5.10 P.M., 100° | 1 in 3000 | 0·5 |
| 84 | 22 | In 1st wave | 98·2, 98·4, 98·4, 98·4, 98·4 99·4, 100·4, 101, 101·6, 101·5 | 16th | 5.0 P.M., 101°·5 | 1 in 2000 | 5·0 |
| 85 | 21 | In 4th wave | 99, 99·4, 99·4, 99, 98·6 101, 101·2, 100, 101, 102 | 118th | 5.10 P.M., 102° | 1 in 1500 | 0·25 |
| 86 | 26 | { In 1st wave of a relapse after normal T. in- terval of 6 weeks } | 99·2, 99·4, 99·4, 99·6, 100·4 102, 101·4, 101, 102·4, 103·2 | 153rd | 5.20 P.M., 103°·2 | 1 in 1600 | 3·00 |
| 87 | 23 | In 1st wave | N. 97, N. 100·2, N. N., N., 101, N., 97·4 | 12th | 10.45 A.M., normal | 1 in 200 | Nil |

| | | | | | | | |
|-----|----|-------------|--|------|--------------------|-----------|--------|
| 88 | 21 | In 1st wave | 101 101·4 100·4 100·2 100·2 102 100·6 101 100·4 101·6 | 23rd | 11.5 A.M., 100°·4 | 1 in 500 | 0·0312 |
| 89 | 32 | In 1st wave | 100·2 101 100·4 100·8 100 101·8 101·6 102·2 101·2 101·6 98·8 98·2 98·2 98·6 98·4 | 21st | 11.15 A.M., 100°·8 | 1 in 40 | Nil |
| 90 | 19 | In 2nd wave | 102·4 101·6 102 101·2 100·2 99·4 98·2 99·4 98 98·4 | 43rd | 4.45 P.M., 100°·2 | 1 in 3000 | 5·00 |
| 91 | 42 | In 2nd wave | 100·8 100·6 102 99·6 99·8 99 100 99·8 100 100·6 | 62nd | 5.0 P.M., 99°·8 | 1 in 500 | Nil |
| 92 | 22 | In 1st wave | 101·4 101 101·4 101·2 99·8 100·2 99·4 101 101·6 101 | 41st | 5.15 P.M., 99°·8 | 1 in 1200 | 0·0156 |
| 93 | 20 | In 1st wave | 101·8 103 102·8 101·4 103 100 101·4 100 100 100·8 | 21st | 4.0 P.M., 103° | 1 in 2000 | 1·00 |
| 94 | 29 | In 1st wave | 102·8 103 102·4 101·6 102·8 98 102 101·2 100·2 100·4 | 31st | 4.15 P.M., 102°·8 | 1 in 2500 | 1·00 |
| 95 | 35 | In 1st wave | 99·8 100 100 100·2 101·4 101 99·6 102·4 102 103 | 21st | 4.30 P.M., 100°·4 | 1 in 3000 | 0·0625 |
| 96 | 36 | In 1st wave | 102·4 98·2 97·2 97·8 98 102·2 98·4 98 97·8 97·8 | 18th | 4.45 P.M., 103° | 1 in 2500 | 0·0312 |
| 97 | 24 | In 1st wave | 102·4 102·2 100·4 102·2 101·2 103 102·6 103 102·4 103·2 | 26th | 4.0 P.M., 97°·8 | ? | Nil |
| 98 | 31 | In 1st wave | 98 99·4 98·6 98·6 98·6 100·2 101·8 101·8 100·2 100·2 | 21st | 4.15 P.M., 103°·2 | 1 in 2500 | 0·0312 |
| 99 | 19 | In 4th wave | 99·8 99·2 99 100·2 101·2 102·2 102·4 102·2 102·5 103·5 | 66th | 4.30 P.M., 100°·2 | 1 in 1000 | 0·0312 |
| 100 | 25 | In 1st wave | 99·2 98·6 99·4 99·2 100 101·6 101 102 103·6 102 | 25th | 4.45 P.M., 103·5 | 1 in 1500 | 0·5 |
| 101 | 25 | In 1st wave | 100·4 101·2 100 99·6 98·8 102·8 102·8 101·8 101·4 100 | 32nd | 4.15 P.M., 102° | 1 in 2400 | 0·25 |
| 102 | 30 | In 1st wave | 100·4 100 2 99·6 99 101 102·2 101·6 101·8 101·6 103 | 22nd | 4.30 P.M., 100° | 1 in 2000 | 0·0625 |
| 103 | 26 | In 1st wave | | 17th | 4.45 P.M., 103° | 1 in 2200 | 1·00 |

| No. of case. | Age. | Stage of the fever. | Temperature of patient for few days preceding bleeding. | Day of disease. | Time of bleeding and patient's temperature. | Maximum dilution of patient's blood giving agglutination. | Recovery of and smallest quantity of blood yielding <i>M. melitensis</i> . |
|--------------|------|---|---|-----------------|---|---|--|
| 78 | 29 | In 1st wave | 98.4, 98.4, 98, 98.4, 99.4 102.2, 102.6, 101.6, 103.4, 103.6 | } 35th | 10.45 A.M., 100° | 1 in 2600 | c.c. 1.00 |
| 79 | 20 | In 1st wave | 98.2, 97.8, 98.4, 98, 97.6 99.6, 101, 100.6, 100, 100.4 | } 18th | 11.0 A.M., 98.4 | 1 in 3000 | 0.125 |
| 80 | 22 | In 1st wave | 98.4, 98.4, 98, 97.4, 97.4 97.6, 97.4, 98, 98.4, 99 | } 17th | 11.10 A.M., 98° | 1 in 2500 | 0.5 |
| 81 | 24 | In 1st wave | 100.2, 98.4, 99, 99, 99 101, 102.4, 99.2, 100, 100.2 | } 12th | 4.40 P.M., 100° 2 | 1 in 500 | Nil |
| 82 | 27 | In 2nd wave..... | 99, 99.6, 98.4, 99.8, 99.2 11.2, 101, 101, 102.2, 101.6 | } 67th | 4.55 P.M., 101° 6 | 1 in 1500 | 1.00 |
| 83 | 37 | In 1st wave | 100.6, 99.4, 100.4, 99, 97.8 102.4, 101.6, 100.6, 100.4, 100 | } 18th | 5.10 P.M., 100° | 1 in 3000 | 0.5 |
| 84 | 22 | In 1st wave | 98.2, 98.4, 98.4, 98.4, 98.4 99.4, 100.4, 101, 101.6, 101.5 | } 16th | 5.0 P.M., 101° 5 | 1 in 2000 | 5.0 |
| 85 | 21 | In 4th wave | 99, 99.4, 99.4, 99, 98.6 101, 101.2, 100, 101, 102 | } 118th | 5.10 P.M., 102° | 1 in 1500 | 0.25 |
| 86 | 26 | { In 1st wave of a relapse after normal T. in- terval of 6 weeks } | 99.2, 99.4, 99.4, 99.6, 100.4 102, 101.4, 101, 102.4, 103.2 | } 153rd | 5.20 P.M., 103° 2 | 1 in 1600 | 3.00 |
| 87 | 23 | In 1st wave | N. 97, N. 100.2, N. N., N., 101, N., 97.4 | } 12th | 10.45 A.M., normal | 1 in 200 | Nil |

| | | | | | | | |
|-----|----|-------------|-------------------------------|------|-------------------|-----------|--------|
| 88 | 21 | In 1st wave | 101 101'4 100'4 100'2 100'2 | 23rd | 11.5 A.M., 100°4 | 1 in 500 | 0·0312 |
| 89 | 32 | In 1st wave | 102' 100'6' 101' 100'4 101'6 | 21st | 11.15 A.M., 100°8 | 1 in 40 | Nil |
| 90 | 19 | In 2nd wave | 100'2 101' 100'4 100'8 100 | 43rd | 4.45 P.M., 100°2 | 1 in 3000 | 5·00 |
| 91 | 42 | In 2nd wave | 101'8 101'6 102'2 98'6 98'4 | 62nd | 5.0 P.M., 99°8 | 1 in 500 | Nil |
| 92 | 22 | In 1st wave | 102'4 101'6 102' 101'2 100'2 | 41st | 5.15 P.M., 99°8 | 1 in 1200 | 0·0156 |
| 93 | 20 | In 1st wave | 99'4 98'2 99'4 98 98'4 | 21st | 4.0 P.M., 103° | 1 in 2000 | 1·00 |
| 94 | 29 | In 1st wave | 100'8 100'6 102' 99'6 99'8 | 31st | 4.15 P.M., 102°8 | 1 in 2500 | 1·00 |
| 95 | 35 | In 1st wave | 99 101'4 101' 101'2 99'8 | 21st | 4.30 P.M., 100°4 | 1 in 3000 | 0·0625 |
| 96 | 36 | In 1st wave | 101'4 101' 101'4 101'6 101 | 19th | 4.45 P.M., 103° | 1 in 2500 | 0·0312 |
| 97 | 24 | In 1st wave | 100'2 99'4 101' 101'6 103 | 26th | 4.0 P.M., 97°8 | ? | Nil |
| 98 | 31 | In 1st wave | 101'8 103 102'8 101'4 103 | 21st | 4.15 P.M., 103°2 | 1 in 2500 | 0·0312 |
| 99 | 19 | In 4th wave | 100 100'4 99 99'2 97'2 | 68th | 4.30 P.M., 100°2 | 1 in 1000 | 0·0312 |
| 100 | 25 | In 1st wave | 98 102' 101'2 100'2 100'4 | 25th | 4.45 P.M., 103°5 | 1 in 1500 | 0·5 |
| 101 | 25 | In 1st wave | 102'4 102'2 103 102'4 103'2 | 32nd | 4.15 P.M., 102° | 1 in 2400 | 0·25 |
| 102 | 30 | In 1st wave | 103 102'6 103 102'4 103'2 | 22nd | 4.30 P.M., 100° | 1 in 2000 | 0·0625 |
| 103 | 26 | In 1st wave | 98 99'4 98'6 98'6 98'6 | 17th | 4.45 P.M., 103° | 1 in 2200 | 1·00 |
| | | | 100'2 101'8 101'8 100'2 100'2 | | | | |
| | | | 99'8 99'2 99 100'2 101'2 | | | | |
| | | | 102'2 102'4 102'2 102'5 103'5 | | | | |
| | | | 99'2 98'6 99'4 99'2 100 | | | | |
| | | | 101'6 101' 102' 102'6 102 | | | | |
| | | | 100'4 101'2 100 99'6 98'8 | | | | |
| | | | 102'8 102'8 101'8 101'4 100 | | | | |
| | | | 100'4 100'2 99'6 99 101 | | | | |
| | | | 102'2 101'6 101'8 101'6 103 | | | | |

Examination of Bloods.

Table showing in chronological order the date of the disease in each of the 103 cases in which blood was taken for bacteriological examination and the result. The word "Nil" means no *M. melitensis* was recovered; the days of disease not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by two to six blood examinations. As stated before, this has been unavoidable; the number of cases willing to submit to venous puncture was too small to admit of selection. The minimal amounts of blood yielding *M. melitensis* in each case are expressed in fractions of a cubic centimetre.

| Day of disease. | Recovery and quantity or no recovery. | Day of disease. | Recovery and quantity or no recovery. | Day of disease. | Recovery and quantity or no recovery. |
|-----------------|---|-----------------|--|-----------------|---------------------------------------|
| 1 | | 37 | $\frac{7}{8}$ c.c. | 75 | |
| 2 | | 38 | | 76 | |
| 3 | | 39 | | 77 | |
| 4 | | 40 | 1 c.c. | 78 | |
| 5 | | 41 | Nil, $\frac{1}{16}$, $\frac{1}{8}$ c.c. | 79 | |
| 6 | | 42 | $\frac{1}{128}$ c.c. | 80 | |
| 7 | $\frac{1}{8}$, $\frac{1}{128}$ c.c. | 43 | 5 c.c. | 81 | |
| 8 | $\frac{1}{64}$ c.c. | 44 | | 82 | |
| 9 | Nil, $\frac{1}{8}$, $\frac{1}{8}$, Nil. | 45 | | 83 | |
| 10 | $\frac{1}{8}$, 1 c.c. | 46 | | 84 | |
| 11 | $\frac{3}{4}$ c.c. | 47 | | 85 | |
| 12 | 1, Nil, Nil. | 48 | Nil. | 86 | |
| 13 | Nil. | 49 | $\frac{9}{10}$ c.c. | 87 | |
| 14 | | 50 | | 88 | |
| 15 | Nil, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{128}$ c.c. | 51 | 2 c.c. | 89 | |
| 16 | Nil, 5 c.c. | 52 | | 90 | $\frac{1}{128}$ c.c. |
| 17 | Nil, Nil, $\frac{1}{32}$, $\frac{1}{16}$, $\frac{1}{8}$, 1 c.c. | 53 | | 91 | |
| 18 | Nil, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ c.c. | 54 | | 92 | |
| 19 | $\frac{1}{8}$, $\frac{1}{32}$ c.c. | 55 | $\frac{1}{64}$, $\frac{1}{64}$ c.c. | 93 | |
| 20 | | 56 | $\frac{1}{4}$, $\frac{1}{8}$ c.c. | 94 | |
| 21 | Nil, 1, $\frac{1}{16}$, $\frac{1}{32}$ c.c. | 57 | Nil. | 95 | $\frac{1}{8}$ c.c. |
| 22 | Nil, Nil, 1, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{16}$ c.c. | 58 | | 96 | $\frac{1}{4}$ c.c. |
| 23 | $\frac{1}{16}$ c.c. | 59 | | 97 | |
| 24 | Nil, $\frac{1}{32}$ c.c. | 60 | | 98 | $\frac{1}{8}$ c.c. |
| 25 | $\frac{1}{128}$, $\frac{1}{8}$ c.c. | 61 | | 101 | Nil. |
| 26 | $\frac{1}{16}$, Nil. | 62 | Nil. | 108 | Nil, Nil. |
| 27 | Nil. | 63 | | 109 | |
| 28 | Nil, Nil. | 64 | | 110 | |
| 29 | | 65 | $\frac{1}{128}$ c.c. | 116 | Nil, Nil. |
| 30 | Nil, $\frac{9}{10}$ c.c. | 66 | $\frac{3}{32}$ c.c. | 118 | $\frac{1}{4}$ c.c. |
| 31 | Nil, $\frac{1}{8}$, 1 c.c. | 67 | 1 c.c. | 120 | $\frac{1}{16}$ c.c. |
| 32 | Nil, $\frac{1}{4}$ c.c. | 68 | | 149 | Nil. |
| 33 | $\frac{1}{4}$ c.c. | 69 | Nil. | 151 | 1 c.c. |
| 34 | $\frac{1}{4}$ c.c. | 70 | $\frac{3}{32}$ c.c. | 153 | 3 c.c. |
| 35 | $\frac{1}{8}$, 1 c.c. | 71 | | 158 | $\frac{1}{4}$ c.c. |
| 36 | 1, $\frac{1}{64}$ c.c. | 72 | | 240 | Nil. |
| | | 73 | | | |
| | | 74 | Nil. | | |

Remarks.—It will be seen from an inspection of the foregoing tables that at any period of this fever up to the commencement of the 6th month of it (158th day) the causal micro-organism may be found in the blood, and in as small a quantity of blood in the course of the 3rd month as in the 1st month.

The smallest quantity of blood from which in this series of 103 observations it has been isolated has been $\frac{1}{256}$ c.c., practically 4 cub. mm., and that only in two cases.

It is unsafe to assume, as one investigator has done, that in any given case the smallest quantity of blood yielding *M. melitensis* contained only one micrococcus. It would be inexact to express the fact that $\frac{1}{256}$ of a c.c. of blood is the smallest quantity of blood yielding *M. melitensis* in a particular case, in the form that this blood contained 256 micrococci per c.c., and equally inexact to state the fact that 0.1 c.c. of blood incubated in 10 c.c. of broth yielded 31 colonies of *M. melitensis* on an agar slope, in the form that this blood contained 310 micrococci per c.c.; or that 0.25 c.c. of blood spread on the surface of an agar in a Petri dish and incubated, yielded 30 colonies of *M. melitensis* as 120 micrococci per c.c. We do not know whether this micrococcus in the blood is free in the plasma, is phagocyted inside a white blood corpuscle, or is present in both these conditions. If inside a leucocyte, we have yet to learn in what period of time the leucocyte can destroy the vitality of the micrococcus. In various experiments made to ascertain the phagocytic power of fresh normal blood on *M. melitensis*, I have frequently seen as many as 20 and 30 micrococci inside one leucocyte, and it is highly improbable that in combining blood with a nutrient medium, the leucocytes are completely fragmented, and any micrococci they may contain evenly distributed through the medium. For these reasons the somewhat cumbersome method of expressing results as minimal quantity of blood yielding *M. melitensis* has been adhered to as having at least the merit of accuracy.

Minimal Quantity of Blood Yielding M. melitensis.

It will be noticed that the minimal quantity of blood necessary to yield *M. melitensis* in these cases varies within very wide limits, from $\frac{1}{256}$ c.c. (Cases 43 and 47) to 5 c.c. (Cases 84 and 90), that is from approximately 4 cub. mm. to 5000 cub. mm. This surely was to be expected; why should it be constant in a series of patients any more than their agglutinating power on *M. melitensis*, which varies from nil to $\frac{1}{3500}$ or $\frac{1}{8000}$; or, indeed, any other clinical phenomenon?

Here seems the most appropriate place to discuss a feature I made mention of in my former report under the name of "skipping"; where *M. melitensis* was found in some of the higher blood dilutions, and absent from some of the lower, these having been "skipped" or "jumped." This occurred in two of the first series of 51 cases, and in

five of the second series of 52 cases. In Case 59 it was recovered only from the broth-tube containing $\frac{1}{8}$ c.c. of blood, all the other tubes remained sterile; excepting the flask containing 3 c.c. of blood here as a total of 2 c.c. of blood was incubated, obviously this had to be reported as a recovery of *M. melitensis* from 2 c.c. In Case 63 *M. melitensis* was recovered only from the 1 c.c. and the $\frac{1}{8}$ c.c. tubes, the others remaining sterile; it is reported as a recovery from 1 c.c. In Case 77 *M. melitensis* was found only in 3 c.c., 1 c.c., and $\frac{1}{8}$ c.c. tubes; it is reported as a recovery from 1 c.c. In Case 84, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the 3 c.c., 1 c.c., $\frac{1}{2}$ and $\frac{1}{8}$ to $\frac{1}{5\frac{1}{2}}$ c.c. tubes remaining sterile; it is reported as a recovery from 5 c.c. In Case 90, *M. melitensis* was found only in the $\frac{1}{4}$ c.c. tube, the others, 3 c.c. to $\frac{1}{5\frac{1}{2}}$ c.c., remaining sterile; it is reported as a recovery also from 5 c.c.

This phenomenon must, I think, be interpreted as resulting from the small quantity of *M. melitensis* in the circulating blood; in these cases it would seem there was not enough to supply the tubes found sterile, and it would be a matter of chance into which tube the small apparently indivisible amount of *M. melitensis* got.

In no case has the minimal quantity of blood experimented with, $\frac{1}{5\frac{1}{2}}$ c.c. (about 2 cub. mm.) ever yielded *M. melitensis*; in only two cases out of the 103 has the minimal quantity of blood been so small as $\frac{1}{25}$ c.c. (4 cub. mm.). This has a most important bearing on the question of the possibility of the transmission of infection by biting insects such as mosquitoes, which is still *sub judice*.

It is a larger quantity of blood than any biting insect to be found in Malta can contain. Again, with the possible exception of plague, no known bacterial disease has yet been proved to be thus conveyed; this mode of conveyance of infection would appear to be confined to protozoal diseases, and Schandinn's recent work on blood parasites,* in which he demonstrates that in the gnat the "indifferent" spirochætes are so small as only to be visible when a number have agglomerated, and that they can pass through a Chamberland filter, tends to place yellow fever, hitherto considered doubtful in this regard, amongst the protozoal diseases.

Is there any Relation between Temperature of the Patient and the Presence of M. melitensis in his Blood?

Taking first the temperatures for the few days preceding and including the date of the abstraction of blood, and grouping these in a tabular form together with the results of the blood examinations, we get the following table:—

* "Generations und Wirtswechsel bei Trypanosoma und Spirochæte," 'Arbeiten aus dem Kaiserlichen Gesundheitsamte,' Band 20, Heft 3, 1904.

| Course of patient's temperature. | No. of cases. | No recovery of <i>M. melitensis</i> . | Recovery of <i>M. melitensis</i> . | Minimal quantities of blood yielding <i>M. melitensis</i> where recovered. |
|---------------------------------------|---------------|---------------------------------------|------------------------------------|---|
| | | | | c.c. |
| Steady between— 98° and 101° | 20 | 7 | 13 | 1, $\frac{1}{10}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 99° and 102° | 22 | 8 | 14 | 1, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 100° and 103° | 20 | 4 | 16 | 3, 2, 1, 1, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |
| 101° and 104° | 12 | 3 | 9 | $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 102° and 105° | 1 | — | 1 | $\frac{1}{2}$ |
| Ascending | 7 | 1 | 6 | 5, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$ |
| Descending | 14 | 6 | 8 | 5, 1, $\frac{1}{10}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$ |
| Steady about normal | 7 | 5 | 2 | $\frac{1}{2}$, $\frac{1}{2}$ |
| Total cases ... | 103 | 34 | 69 | |

Next grouping the cases according to the patient's temperature at the time blood was abstracted, we get:—

| Patient's temperature. | No. of cases. | No recovery of <i>M. melitensis</i> . | Recovery of <i>M. melitensis</i> . | Minimal quantities of blood yielding <i>M. melitensis</i> where recovered. |
|------------------------|---------------|---------------------------------------|------------------------------------|---|
| | | | | c.c. |
| 97° to 97°·9... | 1 | 1 | | |
| 98° to 98°·9... | 20 | 10 | 10 | 1, $\frac{1}{10}$, $\frac{1}{10}$, $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 99° to 99°·9... | 21 | 9 | 12 | 1, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{4}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 100° to 100°·9... | 22 | 7 | 15 | 5, 2, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |
| 101° to 101°·9... | 13 | 5 | 8 | $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |
| 102° to 102°·9... | 15 | 1 | 14 | 5, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |
| 103° to 103°·9... | 11 | 1 | 10 | 1, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |
| Total cases ... | 103 | 34 | 69 | 5, 3, 1, 1, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$, $\frac{1}{2}$ |

It will be seen that both these tables show a distinct relation between the presence of *M. melitensis* in the peripheral blood and the patient's temperature; the first showing an increasing ratio of recoveries of *M. melitensis* with the higher temperatures; the second similarly; but no such relationship is visible between temperature and minimal quantity of blood containing *M. melitensis*.

Is there any Relation between the Agglutinating Power of Blood of Mediterranean Fever Cases and the Presence of M. melitensis therein?

When commencing the summer examination of these bloods in June, 1904, to corroborate the diagnosis of Mediterranean Fever arrived at by the medical officer in charge of the case, I invariably examined some of the blood drawn for agglutination reaction on *M. melitensis*, and the diagnosis of the cases selected and given is as certain as it can be. After doing a few cases, it was felt it would be of interest to ascertain what, if any, relation existed between high or low agglutinative power and the presence of *M. melitensis* in the blood. It was accordingly necessary to fix on an arbitrary standard to which all recorded agglutination reactions in the series should conform, in no matter what dilution of serum. For the purpose of this work it was therefore laid down that no agglutination reaction would be recorded unless visible under the $\frac{2}{3}$ -in. objective of the microscope 15 minutes after contact between diluted serum and emulsion of *M. melitensis* in normal salt solution. The dilutions of the serum were made with a mercury calibrated 5 cub. mm. pipette graduated in $\frac{1}{2}$ cub. mm., the various dilutions and the *M. melitensis* emulsion brought together on a glass slide, which was put in a moist chamber for 15 minutes and then examined under the microscope side by side with a control: and the highest dilution in which agglutination had by then occurred was recorded as the "maximum dilution of patient's blood giving agglutination reaction." It will thus be seen that all these are strictly comparable for both series. This was done for 89 cases out of the 103. It is usual to express a positive agglutination reaction for *M. melitensis* as 1 in "n," meaning that 1 bulk of serum in "n" bulks of normal saline effects agglutination: this may be expressed as a fraction $\frac{1}{n}$,

or again one may say that the agglutinating power of a given patient's serum is "n." In the following table in the column "Agglutinating power," the numbers given mean that one bulk of serum diluted with the corresponding number of bulks of normal saline has sufficed to produce agglutination under the conditions already specified; in the column headed "No *M. melitensis*" is placed the number of cases which did not yield *M. melitensis*; in the column headed "Recovery of *M. melitensis*" is placed the number of cases yielding *M. melitensis*, and in the last column are placed the minimal quantities of blood yielding *M. melitensis* for the specified agglutinating power.

Here one can trace no relation between the amount of agglutinating power and the presence or absence of *M. melitensis* in the blood, but there is some indication of a relationship between agglutinating power and minimal quantity of blood yielding *M. melitensis*, which might be tentatively put as follows:—The higher the agglutinating power of a

| Agglutinating power. | No <i>M. melitensis</i> . | Recovery of <i>M. melitensis</i> . | Minimal quantity of blood yielding <i>M. melitensis</i> where recovered. | Agglutinating power. | No <i>M. melitensis</i> . | Recovery of <i>M. melitensis</i> . | Minimal quantity of blood yielding <i>M. melitensis</i> where recovered. |
|----------------------|---------------------------|------------------------------------|---|----------------------|---------------------------|------------------------------------|--|
| 1 in 20 | 1 | | c.c. | 1 in 1080 | 2 | | c.c. |
| 40 | 5 | 3 | $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{128}$ | 1200 | 2 | 1 | $\frac{1}{8}$ |
| 100 | — | 1 | $\frac{1}{32}$ | 1400 | 1 | 2 | 1, 1 |
| 200 | 1 | 3 | $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{64}$ | 1500 | — | 4 | 5, 1, $\frac{1}{4}$, $\frac{1}{8}$ |
| 300 | — | 1 | 1 | 1600 | — | 2 | 3, $\frac{1}{4}$ |
| 360 | — | 2 | $\frac{1}{4}$, $\frac{1}{8}$ | 1800 | 3 | | |
| 400 | — | 1 | $\frac{1}{64}$ | 2000 | 1 | 7 | 5, 1, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ |
| 500 | 5 | 8 | 1, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$ | 2200 | — | 1 | 1 |
| 600 | — | 4 | 1, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$ | 2400 | — | 1 | $\frac{1}{4}$ |
| 640 | — | 1 | $\frac{1}{8}$ | 2500 | 1 | 4 | 1, $\frac{1}{4}$, $\frac{1}{32}$, $\frac{1}{64}$ |
| 800 | 1 | 5 | $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$ | 2600 | — | 1 | 1 |
| 1000 | 2 | 8 | 2, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$ | 3000 | — | 4 | 5, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ |

blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be ; whence one might be tempted to deduce a correlation between high agglutinating power and high resistance to *M. melitensis* invasion on the part of the patient.

For how long is it Necessary to Incubate in Broth Patients' Blood containing M. melitensis before its Presence can be Demonstrated ?

To obtain an answer to this question, the broth-tube containing the largest quantity of blood was, in 25 cases, used to inoculate, on each of eight successive days after its abstraction, an agar slope which was dated and incubated at 37° C. Seven of these cases failed to yield *M. melitensis*, in the others it made its appearance variably on the slope inoculated 3, 4, 5, 6, 7, or 8 days after commencement of the incubation of the blood in broth as follows :—

| Number of days' incubation. | Number of recoveries. | Minimal amounts of blood ultimately yielding <i>M. melitensis</i> . |
|-----------------------------|-----------------------|---|
| 3 | 3 | c.c. $\frac{1}{8}$, $\frac{1}{4}$, $\frac{1}{2}$ |
| 4 | 3 | 1, $\frac{1}{4}$, $\frac{1}{8}$ |
| 5 | 5 | 1, 1, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{8}$ |
| 6 | 2 | 1, 1 |
| 7 | 4 | 5, 5, $\frac{1}{4}$, $\frac{1}{8}$ |
| 8 | 1 | 8 |

The general inference to be drawn from this is that, in general, the smaller the "minimal" amount of blood, the earlier *M. melitensis* makes a demonstrable appearance in the blood. The question is of some interest, because Widal, in his similar work on typhoid fever, postulated the hypothesis of the existence in the blood of typhoid cases in variable amounts of what he called "substances empechantes," which delayed the growth of *B. typhosus* in his nutrient broth according to the amount thereof ; *B. typhosus*, in his experience, sometimes appearing after one day's incubation, in others not till after eight days. It will be observed that the foregoing cases yield some little support to the theory ; for instance, in one case, where the minimal amount of blood was $\frac{1}{16}$ c.c., *M. melitensis* made its appearance after three days ; in another case, where the minimal amount of blood was the same, $\frac{1}{16}$ c.c., not till the 7th day.

Diurnal Variation.

In 58 of the 103 cases blood was drawn in the forenoon ; in 34 of these cases *M. melitensis* was present, in 24 it was absent ; in 45 cases blood was drawn late in the afternoon ; *M. melitensis* was present in 35 cases, absent in 10 ; a ratio of presence to absence of 7 to 5 in the

morning, as against 7 to 2 in the evening; that is *M. melitensis* was $2\frac{1}{2}$ times more likely to be found in blood taken in the late afternoon than in the forenoon; this suggests a correlation between the usually higher temperature of the patient in the afternoon and the presence of *M. melitensis* in his blood.

Summary.

1. *M. melitensis* has been demonstrated to be present in the peripheral blood of 68 per cent. (2 out of 3) of the cases examined in a series of 103 cases.

2. *M. melitensis* exists in the blood in relatively small amount, not having been found in association with a less quantity of blood than 4 cub. mm., and that only in two cases out of 103.

3. The higher the temperature of the cases for a few days before and at the period when blood is abstracted, the more likely is the latter to contain *M. melitensis*.

4. The higher the agglutinating power of a blood during the fever, the larger is the minimal quantity of blood yielding *M. melitensis* likely to be. A correlation is suggested between this and the patient's powers of resistance to *M. melitensis*.

5. The smaller the minimal quantity of blood yielding *M. melitensis*, the earlier is *M. melitensis* likely to be obtained from the nutrient broth in which it is being incubated.

6. *M. melitensis* is more likely to be present in blood abstracted in the late afternoon than in the forenoon. A correlation is suggested between this and usual evening rise of temperature.

7. No definite relation can be established between any chronological stage of the fever and the presence of *M. melitensis* in the blood; it is present both early and late in the disease.

II. ON THE INFECTIVITY OF THE SKIN, BREATH, AND SWEAT OF MEDITERRANEAN FEVER PATIENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

1. *Examination of Skin.*

The epidermis is considerably affected in Mediterranean Fever. Hughes states that "about the fourth week desquamation occurs, being most noticeable on the soles of the feet, where the skin peels off in large flakes, leaving the skin of the feet soft and tender for a considerable time," "during the fourth month towards the end of long attacks or even during early convalescence, the hair falls out extensively." "In long cases the nails have often a grooved longitudinally striated appearance." Consequently, in March of 1904, I set myself to make an attempt to determine if *M. melitensis* is excreted in the cast-off epidermal scales of the skin. After some consideration I determined to put epidermal scrapings from Malta Fever Patients into nutrient broth, incubate this, and then from it to attempt to isolate *M. melitensis* if present.

As the skin is well known to be, even under apparently healthy conditions, largely infected with various organisms, and as any attempt at sterilisation thereof might kill off the somewhat delicate *M. melitensis*, and so baulk the object in view, it was felt that some other method of restricting the presence of organisms which would inevitably overgrow the slow growing *M. melitensis* during the process of isolation would have to be resorted to, and the addition of some antiseptic to the nutrient broth which would to some extent hold back the usual skin organisms, and yet not unduly check the possible *M. melitensis*, seemed the most promising method. Formalin was the antiseptic ultimately decided upon, and a series of preliminary experiments enabled one to determine that broth (made with peptone-Martin, and of an acidity + 5 Eyre's scale) containing 1 in 1000 of sodium formate would, after inoculation with *M. melitensis*, give a good growth thereof in four days; 2 in 1000 delayed growth till sixth day, which was felt to be too long, and less than 1 in 1000 would have defeated the object in view of restraining other organisms. The procedure finally adopted was the following:—

Some of the surface epidermis was removed from each selected Malta Fever patient with a sterilised scalpel by scraping the surface of the arms, chest, thighs, and feet; these scrapings were placed in a numbered, dated, test-tube containing the broth specified, incubated at

37° C. for five days (thus giving one day's margin), then the broth-tube was well agitated, a loopful taken from it and placed in another tube, of same number but new date, containing 10 c.c. sterile broth of the kind specified, this well agitated and mixed, and from this dilution zig-zag stroke inoculations were made on large agar (+ 5 Eyre's scale) slope tubes of same number and new date, the new tubes incubated for five days, the agar slopes examined daily for discrete colonies, which never failed to appear, and all of these resembling *M. melitensis* colonies were subjected to the usual tests; the second broth-tube used to form a similar dilution for third generation in broth and on agar slopes, and these again for Fourth Generation.

A total of 14 patients (the opportunity for obtaining material from whom I owe to the kind courtesy of the officers R.A.M.C. of Valletta and Cottonera hospitals, to whom I beg to tender my warmest thanks), all of which cases were undoubted Mediterranean Fever, ranging from three weeks to three months in duration of disease, were thus examined during April, May, and June, 1904. Every case yielded discrete colonies on the agar slopes, many of them greatly resembling *M. melitensis* colonies at first sight, but proving on further examination to be a white staphylococcus, apparently Welsh's skin staphylococcus, and not one of them turned out to be a colony of *M. melitensis*.

In August the foregoing method was modified as follows, the broth enrichment method being abandoned:—The epidermal scrapings from each patient were thoroughly ground up in 1 c.c. of sterile normal salt solution, one loopful of this was used to plate three successive glucose-litmus-agar Petri dishes; the remaining epidermal emulsion was diluted by the addition of 5 c.c. more normal saline, and the surface of three other similar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted epidermal emulsion over each, and the six plates then incubated at 37° for five days, at the end of which they were carefully examined for possible colonies of *M. melitensis*, and all likely looking ones put through the usual tests. A total of 71 specimens were thus examined. The accompanying table shows the cases and the day of disease on which specimens were taken, the sign × indicating each examination. The first 14 cases are not included, the day of disease not having been sufficiently accurately recorded.

From none of these bacteriological examinations has *M. melitensis* ever been recovered, but in nearly every plate out of the 426 used in this investigation has been found most constantly a Gram staining glucose fermenting white staphylococcus, presumably the same described by Welsh as associated with the skin, and on taking off the covers of the Petri dishes, a faint sour odour very similar to that noticeable on raising the bed-clothes of a feverish rheumatic patient was generally perceptible, suggesting the possibility of "sour sweats" being due to fermentations set up by this organism.

Table showing Cases and Day of Disease on which Skin was examined Bacteriologically—continued.

[illegible]

Animal Experiments with Skin Scrapings.

It was necessary to see also whether any evidence of the presence of *M. melitensis* in the skin of patients could be obtained by animal experimentation, and accordingly the following were undertaken:—

Experiment I.—Monkey No. 54.

A freshly arrived animal whose temperature and whose blood gave not the slightest indication of reaction to *M. melitensis* was set apart for this experiment.

July 21. Epidermal scrapings from the arms, forearms, and flanks of six Malta Fever patients, all between 28th and 35th day of disease, were taken, ground up in 5 c.c. sterile nutrient broth in a small sterilised mortar with a sterile pestle, and the resulting emulsion injected subcutaneously with a sterile syringe between the animal's shoulders, the intention being to first get general evidence, and later work out details.

July 22. Evening temperature had risen to 104° F.

July 24. Injection not yet showing any indication of absorption; persists as a globular swelling.

July 25. Some indication of commencing suppuration near site of injection. Evening temperature 104° F.

July 27. Commencing suppuration of 25th has now aborted. Injection still persists as a globular swelling.

July 29. Injectional swelling now disappearing.

July 31. No trace of agglutination in a dilution of $\frac{1}{20}$.

August 3. Temperature has remained normal since July 24. Again made emulsion of the skin-scrapings from five patients all between 30th and 45th day of disease, and injected subcutaneously.

August 5. No swelling, but some induration at sight of last injection.

August 7. No trace of agglutination reaction in a dilution of $\frac{1}{10}$. This monkey's box is next to that of Monkey No. 55, which, unknown to me, had been artificially infected with Malta Fever, and contact was possible (see note at end of this experiment).

August 9. Evening temperature to-day 104°, though normal from July 26 to this morning.

August 10. Agglutination reaction is present in a dilution of $\frac{1}{10}$ visible to naked eye, in $\frac{1}{20}$ visible under $\frac{2}{3}$ -in. objective.

August 11. Temperature again normal. Again repeated injection of epidermal scrapings from four other cases of Malta Fever, all between 30th and 45th day of disease.

August 15. Agglutination reaction present in a dilution of $\frac{1}{10}$ visible to naked eye, and of $\frac{1}{20}$ visible under $\frac{2}{3}$ -in. objective.

August 26. Agglutination reaction present as on August 15.

September 5. Agglutination reaction visible to naked eye in a dilution of $\frac{1}{20}$, and in $\frac{1}{40}$ under $\frac{2}{3}$ -in. objective.

September 28. Agglutination reaction as on September 5.

October 1. Agglutination reaction as on September 5. There has been no rise of temperature since August 9. Monkey killed with chloroform, an aseptic *post-mortem* made, and two broth-tubes and one agar slope inoculated with small cubes of tissue from the spleen, the broth-tubes each receiving a piece of the organ; similarly the liver and kidney, and all incubated. All organs were healthy, and there was no enlargement of the spleen.

October 8. No growth in any slope of October 1. Now inoculated six slopes from the six broth-tubes of October 1.

October 13. No growth in any slopes of October 1 or October 8. Experiment concluded.

Note.—As mentioned above, this monkey had been unwittingly exposed to the possibility of contact infection from Monkey No. 55, but as the animal cannot be safely said to have developed the fever, this does not matter. It had an occasional rise of temperature, but lasting only a day. No *M. melitensis* was recovered *post-mortem*, and an agglutination of $\frac{1}{40}$ alone is insufficient on which to base a diagnosis, and I should consider the development of this reaction due to the injection of *M. melitensis* toxins (contained in the skin). Of the action of toxins in producing the agglutination reaction I give experimental evidence in another section of this Report.

Experiment II.—Monkey No. 68.

During the first weeks of the last experiment the possibility of excretion of *M. melitensis* in the urine became an established fact, and as then the possibility of patients infecting the skin of their flanks with their own urine had to be considered, it was resolved that henceforth only scrapings from the upper arms of patients should be used. Further, the monkey (No. 68) used for this second experiment had his box put in a corner with no neighbour on his left, and the monkey used in the preceding experiment on his right, and as the latter did not develop the fever, and No. 68 was within reach of no other, he must be considered as not having been exposed to the risk of contact infection.

Monkey No. 68 was kept under observation from August 12 to 20; during this period his temperature varied from 100° — $103^{\circ} \cdot 2$; blood presented no trace of agglutination reaction with *M. melitensis*.

August 20. Epidermal scrapings from upper arms of five patients, all between 30th and 60th day of disease, were emulsified as before, and injected between shoulders subcutaneously.

August 26. There has been no fever since last note, and to-day there is no trace of agglutination reaction.

August 27. Second injection of arm scrapings from two patients, both in 91st day of disease.

September 2. Third injection of arm scrapings of three patients, all between 60th and 90th day of disease.

September 5. Agglutination reaction present in a dilution of $\frac{1}{40}$, not beyond.

September 9. Ill, and off his feed.

September 10. Died between 5 and 7 P.M. In my absence an immediate *post-mortem* was made by Major Horrocks, who notes: "Very emaciated, maggots on skin of face, spleen and kidneys appeared slightly congested, other viscera appeared normal. Made cultures from spleen and kidney."

September 19. Agar slopes inoculated from spleen on 10th have remained sterile. Kidney slopes planted from broth on 14th also sterile; reinoculated these.

September 20. Reinoculated spleen slopes.

September 25. All remain sterile.

Result.—No development of fever, but development of a low ($\frac{1}{40}$) agglutination reaction, probably the result of injection of *M. melitensis* toxins in the epidermal scrapings. No *M. melitensis* recovered *post-mortem*.

Experiment III.—Monkey No. 62.

This experiment was commenced by Major Horrocks, and was turned over by him to me to complete on September 28, just prior to his departure for England. For convenience of comparison, I will briefly recapitulate Major Horrocks' notes.

Monkey No. 62 had had its blood frequently examined during August, and up to commencement of the experiment, and its temperature had been daily recorded. It was absolutely free from any suspicion of Malta Fever.

September 16. Monkey No. 62 received an injection of skin scrapings from arms and axilla of a fever patient, ground up in normal saline solution.

September 21. Blood examined, no agglutination reaction to *M. melitensis*.

September 24. Skin scrapings from same patient again injected.

September 26. Blood again examined. No agglutination reaction to *M. melitensis*.

September 27. Skin scrapings from same patient again injected.

September 30. This morning monkey was too sick to have his temperature taken. Died at 10.30 A.M. *Post-mortem* made at once. All organs seemed healthy. No cause of death discoverable. Inoculated broth-tubes and agar slopes from spleen, kidney, liver and heart's blood and incubated.

October 7. No growth on any slope of September 30. Incubated fresh slopes from broth-tubes of September 30.

October 12. No growth on any slope of October 7. Experiment concluded.

Result.—No development of Malta Fever. No development of agglutination reaction. No recovery of *M. melitensis*, *post-mortem*.

Note.—This experiment differs from the other two preceding it only in the non-appearance of the agglutination reaction; but, as in Experiment I, Monkey No. 54, the first skin injection was given July 21, and the agglutination reaction did not appear till August 10, an interval of 21 days, nor in Experiment II, Monkey No. 68, till after an interval of 16 days; nor in Experiment IV, Monkey No. 74, next to be described, till after an interval of 22 days; nor in Experiment V, Monkey No. 65, to be described next but one, till after an interval of 23 days; I think one is entitled to consider that as Monkey No. 62, Experiment III, died 14 days after its first skin injection, an interval shorter than any of those just cited, that there had not been time for the agglutination reaction as observed in the others, to develop.

Experiment IV.—Monkey No. 74.

This experiment, like the last, was commenced by Major Horrocks on September 12, during my absence, and turned over to me on September 28 to complete. Blood had prior to experimentation been frequently examined, but had never exhibited the slightest agglutination reaction with *M. melitensis*. There was never any possibility of contact infection.

September 12. Injection of emulsified skin scrapings from arms and axilla of one Mediterranean Fever patient.

September 17. Blood examined. Serum in a low dilution appeared to have a tendency to agglutinate *M. melitensis*.

September 23. Blood again examined. Serum in a dilution of 1 in 10 showed no sign of agglutinating *M. melitensis* after contact of one hour.

September 25. Second injection of skin scrapings emulsified in normal saline.

September 27. Third injection of skin scrapings.

September 28. Blood examined. No agglutination reaction.

October 3. Fourth injection of skin scrapings.

October 4. Blood gives a distinct agglutination reaction in a dilution of $\frac{1}{40}$ in 15 minutes visible under $\frac{2}{3}$ -in. objective.

October 8. Fifth injection of skin scrapings from three patients.

October 11. Blood gives distinct agglutination reaction in a $\frac{1}{80}$ dilution after 15 minutes visible to naked eye.

October 15. Sixth injection of skin scrapings from same three patients.

October 18. Agglutination reaction visible to naked eye in $\frac{1}{80}$ dilution.

October 19. Seventh injection of skin scrapings from three patients.

October 22. Eighth injection of skin scrapings from two patients.

October 25. Agglutination reaction in $\frac{1}{40}$ dilution visible to naked eye and in $\frac{1}{80}$ visible under $\frac{2}{3}$ -in. objective.

October 26. Ninth injection of skin scrapings from four patients.

October 28. This morning this monkey was found dead. *Post-mortem*. Stomach much dilated with gas. Organs all apparently healthy, much muscular wasting, abscesses at sites of injections. Broth-tubes and agar slopes inoculated from all organs and incubated.

November 2. Agar slopes of October 28 all sterile. Inoculated fresh agar slopes from broth tubes and incubated.

November 7. Agar slopes of November 2 sterile, as are also those of October 28. Experiment concluded.

Result.—Injection of skin scrapings has not been followed by fever, but has developed an agglutination reaction in the serum in low dilution appearing after an interval of 22 days from date of first injection.

Experiment V.—Monkey No. 65.

This monkey had been bitten in September by supposedly infected mosquitoes, but had never had any fever, nor had its blood, which with the others had been examined as a routine measure, once a week, ever shown any sign of agglutinating power on *M. melitensis*.

October 30. This monkey received an injection of epidermal scrapings from arms of four Mediterranean Fever patients.

November 3. Second injection of skin scrapings from four patients.

November 6. Third injection of skin scrapings from four patients.

November 7. Agglutination reaction = a slight tendency only visible in $\frac{1}{10}$, $\frac{1}{20}$ and $\frac{1}{40}$ dilutions, insufficient to call positive.

November 10. Fourth injection of skin scrapings from arms of four patients.

November 13. Fifth injection of skin scrapings from arms of four patients.

November 15. Agglutination reaction = nil in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, and $\frac{1}{40}$.

November 16. Sixth injection of skin scrapings from arms of four patients.

November 19. Seventh injection of skin scrapings from arms of four patients.

November 22. Agglutination reaction distinct traces in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions only.

November 24. Eighth injection of skin scrapings from arms of four patients.

November 27. Ninth injection of skin scrapings from arms of four patients.

November 28. Agglutination reaction marked in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions.

December 1. Tenth injection of skin scrapings from arms of four patients.

December 4. Eleventh injection of skin scrapings from arms of four patients.

December 5. Agglutination reaction only in $\frac{1}{10}$ dilution visible under $\frac{2}{3}$ -in. objective.

December 12. No agglutination reaction.

December 19. No agglutination reaction.

December 26. No agglutination reaction.

January 3. No agglutination reaction.

January 9. No agglutination reaction.

January 16. No agglutination reaction.

January 23. No agglutination reaction.

Experiment concluded. Monkey used for other experiments.

Result.—Infection of skin scrapings has not been followed by fever, but has developed in the blood a low agglutination reaction on *M. melitensis* after an interval of 23 days.

Remarks.—Summarising in tabular form the foregoing five experiments we get the following (see Table, p. 30).

In none of these monkeys can Malta Fever be said to have developed. The appearance of a low agglutination reaction alone is not sufficient on which to base a diagnosis and may, I think, safely be attributed to the presence of *M. melitensis* toxins in the skin scrapings used. It is to be noted that the highest dilution in which it was obtained was $\frac{1}{80}$; this is identical with the highest dilution which the agglutination reaction was obtained in Monkeys 58 and 59, which each received injections of the filtrate from broth in which *M. melitensis* had been cultured (*vide* toxin experiments), and both contrast markedly with the high dilution $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{3000}$, $\frac{1}{3500}$ in which agglutination is quite usually obtainable in monkeys which have received living *M. melitensis* in any experimental manner. The occasional elevations of temperature are attributable to the presence of staphylococci in the skin scrapings used, which had a decided tendency to produce abscess formation.

The point with regard to the toxins was felt to be an important one, and accordingly an effort was made to resolve it by an experiment which was commenced by Major Horrocks on September 22 and continued by me from September 28 to its conclusion on December 26.

| Experiment number. | Monkey number. | Different samples of epidermis. | Number of injections. | Date of first injection. | Date of appearance of agglutination reaction. | Number of days required to develop agglutination. | Highest dilution which gave agglutination. | Post-mortem examination for <i>M. melitensis</i> . |
|--------------------|----------------|---------------------------------|-----------------------|--------------------------|---|---|--|--|
| I | 54 | 15 | 3 | July 21 | Aug. 10 | 20 days | $\frac{1}{30}$ | Not recovered. |
| II | 68 | 10 | 3 | Aug. 20 | Sept. 5 | 16 " | $\frac{1}{40}$ | Not recovered. |
| III | 62 | 3 | 3 | Sept. 16 | Never appeared. Died Sept. 30 | ... | ... | Not recovered. |
| IV | 74 | 18 | 9 | Sept. 12 | Oct. 4 | 22 days | $\frac{1}{80}$ | Not recovered. |
| V | 65 | 44 | 11 | Oct. 30 | Nov. 22 | 23 " | $\frac{1}{30}$ | No post-mortem. |

Experiment VI.

Monkey No. 61A. Had never had any elevation of temperature and had never presented agglutination reaction.

September 22. Skin scrapings from arms and axillæ of two Malta Fever patients were ground up with sterile normal salt solution into a fine emulsion. A sterile Berkefeld candle was fitted to a sterile test-tube, the emulsion filtered, and the filtrate injected subcutaneously into Monkey No. 61A.

September 24. Sweat obtained from three Malta Fever patients, similarly filtered and filtrate injected.

September 26. Blood presented no agglutination reaction.

October 3. Blood presented no agglutination reaction.

October 6. Injected filtered sweat from one patient.

October 8. Skin scrapings from three patients ground up in 15 c.c. sterile salt solution, allowed to macerate at laboratory temperature, 68° F., for two hours, filtered through Berkefeld candle and filtrate injected.

October 10. Blood presented no agglutination reaction.

October 15. Skin scrapings from three patients treated as on October 8 and filtrate injected.

October 17. Blood presented no agglutination reaction.

October 19. Skin scrapings and sweat from four patients treated as on October 8, and filtrate injected.

October 22. Skin scrapings and sweat from three patients treated as on October 8, and filtrate injected.

October 24. Blood presented no agglutination reaction.

October 26. Skin scrapings from four patients treated as on October 8, and filtrate injected.

October 30. Skin scrapings from four patients treated as on October 8, and filtrate injected.

October 31. No agglutination reaction.

November 3. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 6. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 7. Blood presented no agglutination reaction.

November 10. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 13. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 14. Blood presented no agglutination reaction.

November 16. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 19. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 21. Blood presented no agglutination reaction.

November 24. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 27. Skin scrapings from four patients treated as on October 8, and filtrate injected.

November 28. Blood presented no agglutination reaction.

December 1. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 4. Skin scrapings from four patients treated as on October 8, and filtrate injected.

December 26. Blood has been examined weekly since last note and also to-day and has never presented any agglutination reaction with *M. melitensis*.

Experiment concluded.

Remarks.—This experiment, so far as it goes, would appear to negative the explanation given of the appearance of the agglutination reaction in four out of the five preceding experiments, but it is to be observed that it takes for granted one important point, *i.e.*, that *M. melitensis* "toxins" are soluble in normal salt solution. This point is reserved for future experiments, as it does not affect the main inference to be drawn from this series of experiments.

Conclusion.—The active infective agent of Mediterranean Fever is not excreted by the skin.

2. *Examination of Breath.*

With a view to obtaining experimental evidence on this question it was determined to instruct patients to gently blow through sterile broth contained in sterile tubes and examine this broth bacteriologically. Broth tubes were fitted with rubber corks bored with two holes, through one of which was passed a long glass entry tube, bent outside the broth-tube at an obtuse angle of about 150°, length outside broth-tube being about 3 inches, inside dipping beneath surface of broth; through the other hole in the rubber cork was passed a short straight glass air-exit tube lightly plugged with sterile cotton wool 1-inch in length on each side of the rubber cork. The patients whose breath it was desired to examine, were instructed to blow gently down the long entry tube at frequent intervals during one hour, their expired air gently bubbling through the broth. In experiments made before the present series the entry tube outside the broth-tube was fitted with a longish piece of rubber tube and a glass mouth-piece, but it seemed to me that if expired air contained any microbes there was great risk of these being caught by the moist inner surface of the rubber tube, on which a considerable proportion of the water vapour contained in all expired air was found to condense.

First Method.—These broth-tubes on arrival at the laboratory had

their rubber corks and glass tubes removed, were replugged with sterile cotton wool, incubated for seven days at 37°, and then one loopful from each distributed over the agar surfaces of two large-sized Petri dishes, which were incubated for five days at 37°, and then examined for individual *M. melitensis* colonies. A total of 86 such breath-tubes were examined in the manner described, but *M. melitensis* never appeared in any plate, though other organisms, not examined in detail, did.

Second Method.—At this stage other experiments on the vitality of *M. melitensis* growing with other organisms (see Section on Vitality of *M. melitensis* outside the Body, p. 43) had shown that *M. melitensis* had not much chance of surviving in a fluid nutrient medium with other microbes. Accordingly from each broth-tube immediately on its arrival at the laboratory, after well shaking, a loopful was taken and distributed over the agar surface of two Petri dishes, the tubes then plugged with sterile cotton wool and both tubes and plates incubated. At the end of five days these “direct” plates were carefully examined for *M. melitensis*, and at the end of seven days’ incubation a loopful from each breath broth-tube was distributed over two agar plates and these also examined after five days’ incubation for possible *M. melitensis*. One thus had two series of plates, one direct, the other after the incubation of the breath-infected broth. A total of 24 breath-tubes were treated in this manner, but no *M. melitensis* ever appeared on any plate, though the same type of other organisms as before were found.

Third Method.—By this time experiments with *M. melitensis* in association with other organisms had shown that a period of three days was as long as one could expect to recover *M. melitensis* when incubated in broth with other organisms. Accordingly the “direct” series of plates was continued as before, but the breath broth-tubes were only incubated for three days and then plated.

I examined 115 breath broth-tubes in this way, but not in one did I find a single *M. melitensis* colony.

A total of 225 such broth-tubes were examined, and in connection therewith 728 agar Petri plates were prepared and examined, all without result so far as the particular quest involved was concerned. I append a table showing names of patients and day of disease in each case in which breath broth-tubes were prepared, which is indicated in each case by the sign ×. It will be seen that practically the whole period of the disease has been covered.

In order to further investigate the possibility of the infection of Malta Fever being given off in the breath, animal experimentation was also resorted to; portions of such of the foregoing broth-tubes as presented growth being injected in quantities usually of 10 c.c. into two monkeys. This was commenced in the first monkey, No. 73, by Major Horrocks, September 16, and continued by me from September 28 till this monkey's death on November 1; in the second monkey, No. 43, commenced and concluded by me alone.

Monkey No. 73.—This animal had never been used for any other experiment.

September 15. Blood examined. No reaction to *M. melitensis*.

September 16. Injected contents of broth-tube infected by Malta Fever patient's (Lawrence) breath in which growth had occurred after incubation.

September 21. Similar injection (Silburn).

September 28. Blood examined. No reaction to *M. melitensis*.

October 3. Big abscess at site of former injections. To-day injected a growth in breath broth-tube (Anderson) on opposite side.

October 4. Blood examined. No reaction to *M. melitensis*.

October 6. Injected broth growths from breath of Rentcome and Marchant, 5 c.c. from each.

October 9. Similar injection (Silburn).

October 11. Blood examined. Agglutination reaction with *M. melitensis* in $\frac{1}{40}$ dilution under $\frac{1}{8}$ -in. objective.

October 12. Injected broth growths from breaths of Campbell, Joyce and Silburn, 3 c.c. each.

September 16. Injected broth growths from breaths of Campbell, Grimwood and Kinsella, 3 c.c. each.

October 18. Blood presents agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 19. Injected broth growth from breaths of Campbell and Grimwood, 5 c.c. of each.

October 23. Injected broth growths from breaths of Campbell, Kinsella, and Joyce, 3 c.c. of each.

October 25. Agglutination reaction in a dilution of $\frac{1}{100}$ visible to naked eye.

October 26. Injected broth growths from breaths of Fletcher, Groom, Russell and Tait, $2\frac{1}{2}$ c.c. from each.

October 29. Monkey ill. Considerable diarrhoea and wasting.

November 1. Monkey obviously dying. Euthanasia cum chloroform. *Post-mortem.*—Much wasting, no obvious cause of death, organs all apparently healthy, gas in intestines. Agar slopes and broth-tubes inoculated from all organs and incubated.

November 6. Agar slopes of November 1 all sterile. Inoculated fresh slopes from broth-tubes of November 1.

November 11. Agar slopes of November 6 also sterile.

Experiment concluded.

Monkey No. 43.—This animal, in July, on the 16th and 18th, had received injections of the filtrate through filter paper of supposedly infected soil macerated in sterile water, but had never developed Malta Fever, nor had its blood ever reacted to *M. melitensis* in any dilution whatever, though frequently examined.

October 3, 10, 17, 24. No trace of agglutination reaction in dilutions of $\frac{1}{10}$, $\frac{1}{20}$, or $\frac{1}{40}$.

October 27. Injected broth growth from breaths of Grimwood, Joyce and Silburn, 3 c.c. from each.

October 31. Injected broth growth from breaths of Donovan and Silburn, 5 c.c. from each. No agglutination reaction in $\frac{1}{10}$, $\frac{1}{20}$ or $\frac{1}{40}$ dilutions.

November 4. Injected broth growth from breaths of Groom and Silburn, 5 c.c. each.

November 7. A doubtful tendency to agglutination in $\frac{1}{10}$, $\frac{1}{20}$ dilution under $\frac{1}{8}$ -in. objective. Abscess at the site of last injection but one.

November 8. Injected broth growth from breaths of Turner and Kinsella, 5 c.c. each.

November 12. Monkey somewhat ailing.

November 14. No agglutination reaction.

November 17. Injected broth growth from breath of Grimwood, 10 c.c.

November 21. Injected broth growth from breaths of Dennis and Grimwood, 5 c.c. each.

November 21. No agglutination reaction.

November 28. Slight agglutination reaction in $\frac{1}{10}$ and $\frac{1}{20}$ dilutions under $\frac{2}{3}$ -in. objective.

December 1. Injected broth growth from breaths of Darby and Walker, 5 c.c. each.

December 4. Injected broth growth from breaths of Darby, Walker, and Turner, 5 c.c. each.

December 5. Tendency to agglutination in $\frac{1}{10}$ dilution.

December 12. No agglutination reaction. Very weak, thin and emaciated; has been seedy for some days.

December 15. Dying. Gave chloroform. *Post-mortem*, found pneumonia and pericarditis left side. Inoculated slopes and broth-tubes from all organs.

December 23. No *M. melitensis* recovered *post-mortem*, but a glucose fermenting + Gram-staining coccus was obtained from spleen, liver and kidney; nothing from heart's blood and lungs.

Remarks.—There is to be noticed in both these animal experiments the development of a low agglutination reaction, and here as in the

skin experiments I should attribute this to the ingestion of *M. melitensis* toxins, as I consider it practically certain that in breathing out through the broth-tubes, a certain amount of saliva trickled down the long entry tube and so into the broth; the possibility of this was considered at the time and efforts were made to arrange some method of passing breath containing possibly *M. melitensis*, while excluding saliva; but none free from objection was found, hence it was decided to proceed as described, and if *M. melitensis* were obtained, to examine the saliva of patients independently for this micro-organism. In neither monkey could it be said that Mediterranean Fever was developed. The agglutination reaction developed was much too low, the occasional rises of temperature observed were attributable to abscess formation, or to other micro-organisms, not *M. melitensis*, contained in the broth growths which were injected. In both cases temperatures were taken morning and evening all through the experiments.

3. *Examination of Sweat.*

Critical sweats are a not infrequent and quite characteristic feature of Mediterranean Fever, and it has been often felt that it was not impossible that the *Micrococcus melitensis* might be passed out of the body in this secretion. To determine this exhaustively, I made a bacteriological examination of 251 specimens of sweat obtained from patients in the Military Hospital at Valetta. The method adopted was varied from time to time, as will be described.

First Method.—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry, 160° C. in air) lint, placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth-tube numbered, dated, and incubated at 37° C. After five days' incubation, agar slopes inoculated zig-zag from each, were incubated at 37° C., and examined daily for growth; if sterile, original broth-tubes were inoculated with *M. melitensis*, returned to incubator for four days, and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface.

Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

Second Method.—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The

collection was done by the sisters in the ward, who were supplied with the pipettes ready for use, and instructed how to break the point and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

Third Method (a modification of the first).—Circles of lint were obtained saturated with critical sweat from Malta Fever patients as in first method, but instead of being incubated in broth-tubes, were placed each in a 5 c.c. sterile normal salt solution tube, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading $\frac{1}{2}$ c.c. of it over whole surface, and by describing a centripetal spiral with a loopful of the fluid. Discrete colonies were always thus obtained after incubation at 37° C. The critical sweat of seven patients have been thus examined without *M. melitensis* having been obtained.

Fourth Method.—The circles of lint saturated with sweat obtained as in the first method were each placed in a separate broth-tube which was incubated at 37° C. for seven days; then a loopful was taken and placed on the surface of nutrose-glucose-litmus-agar in a Petri dish and spread over it by means of a Klein's platinum spreader, which, after completely going over the agar surface, was straightway passed over the surface of a second similar Petri plate. These plates were then incubated for five days, after which they were examined carefully for possible *M. melitensis* colonies. A total of 81 specimens were thus examined.

Result.—No *M. melitensis* were recovered.

Fifth Method (a modification of the third).—It seemed not unlikely that, supposing *M. melitensis* to be present in the circles of lint saturated with critical sweat, that it would be more likely to be obtained directly without previous incubation if nutrient broth were used instead of the salt solution, specified in Method 3; so, accordingly, the circles of lint were placed in separate broth-tubes, and thoroughly stirred up and agitated therein with a sterile Klein's spreader, followed by a vigorous shaking. Then one platinum loopful was immediately spread over an agar plate and incubated for five days at 37°, and then examined for possible *M. melitensis* colonies. The broth-tubes containing the sweat-saturated lint were incubated seven days, and then one loopful spread over two agar plates. These similarly incubated five days and then examined.

A total of 24 specimens were thus treated, but no *M. melitensis* was ever obtained on either series of plates.

Sixth Method.—At this stage of the examination the results of other experiments (see Section on Vitality of *M. melitensis* outside the Body) indicated that *M. melitensis* could not be expected to be recovered

after three days in a nutrient medium containing other organisms ; so consequently the procedure of the fifth method was modified by reducing the period of incubation of the sweat-saturated lint in broth to three days instead of seven, being otherwise identical.

A total of 30 specimens were so examined, but again no *M. melitensis* was ever obtained from either the plates inoculated the day the specimen was received, or from those inoculated from the broth-tubes after three days' incubation.

Seventh Method.—Instead of grinding up and shaking the sweat-saturated piece of lint in nutrient broth, and immediately plating a loopful of this, the piece of lint as received was placed flat on the agar surface of a Petri dish and pressed well on to the agar with a sterilised Klein's spreader, then it was lifted up with a pair of sterilised forceps and removed with the same surface downwards to the next adjacent area of agar, and there again pressed on to the agar ; this process was repeated until the whole of the agar surface of the Petri dish was covered with "impressions" made from one surface of the piece of lint, usually 30 to 40 for a 10-centimetre Petri. Now another agar Petri was taken, and the same process repeated, but with the other surface of the lint, and both Petri dishes put in incubator at 37° C. ; this completed, the piece of lint was then put in a broth-tube and incubated three days at 37° C., and then one loopful plated over two agar Petris.

A total of 86 specimens were thus examined, but no *M. melitensis* was ever obtained either from the direct series of "impression" plates or from the broth-tubes containing the circles of sweat-saturated lint. Frequently in the course of the examination one met with the colonies of *plus* Gram-staining glucose fermenting staphylococci, which turned up almost invariably in these bacteriological examinations of the skin.

The accompanying table (pp. 41, 42) shows the patients and the day of disease on which a specimen was taken ; it will be seen that practically every day of the disease is represented by one or more examinations. The numbers on the top line indicate the day of disease, one column being given to each ; the sign \times indicates that an examination was made, being placed in the vertical column of the day of disease, and in the horizontal column appropriated to the name of the patient from whom it was taken.

It was found practically impossible to obtain sweat in such quantity as to admit of satisfactory injections into animals, but the number of specimens (251) examined, covering every period of the disease, and the varying methods employed, some of which succeeded so admirably in the isolation of *M. melitensis* from the blood and the urine of patients, practically justify the assumption that *M. melitensis* is not excreted in sweat of Malta Fever patients, or it would have been recovered in one of these numerous attempts.

Result.—*M. melitensis* has not been recovered from the 251 specimens of sweat examined, and in all probability is not excreted in this secretion.

Table showing Cases and Day of Disease on which Sweat was Bacteriologically Examined—*continued.*

| Patient's name. | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | |
|-----------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|-----|-----|--|
| Pigott..... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Wilson | x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hagger | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kelly | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Markham .. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mayes | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Francis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lawrence ... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Martin | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Jones | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hewett | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pudney | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Curry..... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Vincent | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Rivers | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hurrell | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anthony | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Marchant .. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Silburn | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kinsella ... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Turner | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Anderson ... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Dennis | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Derby..... | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ericson | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

III. ON THE VITALITY OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE BODY IN DIFFERENT ENVIRONMENTS.

By Staff-Surgeon E. A. SHAW, R.N., Member Mediterranean Fever Commission, Malta.

In attempting to ascertain the presence or otherwise of *M. melitensis* in the skin, sweat, breath, etc., of patients suffering from Malta Fever, it was obviously of some importance, having regard to the slow growth of this organism, as compared with the rapid growth of others in nutrient broth, a medium which could not be dispensed with, to ascertain for how long it could be recovered when incubated in broth in association with other micro-organisms, and accordingly the following experiments were undertaken, a control inoculation of the *M. melitensis* used being made into a tube of the same broth to verify its viability, the same generation of *M. melitensis* being used throughout.

A. *Vitality of M. melitensis in Mixed Broth Culture.*

No. 1. A sterile broth tube was inoculated with *M. melitensis*, and also from a broth tube (similarly with the same platinum loop) which had been allowed to become contaminated by exposure in the laboratory. This tube was well shaken and then one loopful was distributed over the surface of three successive agar Petri dishes with a Klein's spreader, and then tube and plates placed in incubator at 37°; each day another series of three plates was similarly inoculated from the broth tube, which was each time returned to the incubator, and after a period of five days' incubation, each set of three Petris was carefully examined for *M. melitensis*. This was found in the plates inoculated from the mixed broth culture after it had been incubated seven days, but not later.

No. 2. A repetition of No. 1. The result was the same. *M. melitensis* was recovered from the mixed culture for seven days, but no longer, in both cases the mixed culture at the termination of the experiment, 15th day of incubation, was slightly alkaline to litmus.

Nos. 3 and 4. On the same lines as No. 1; but in these two the mixed culture was composed of *M. melitensis* plus organisms derived from sweat, skin, and urine of Malta Fever cases, as far as possible equal quantities of each being taken. In No. 3, *M. melitensis* was recovered after two days' incubation, not later. In No. 4, started a week later, but with skin, sweat, and urine organisms from different sources, *M. melitensis* was not recovered at all. In both the reaction of the

mixed broth culture was acid to litmus at the termination of the incubation (seven days).

Nos. 5, 6, 7, 8, 9, and 10. In each of these, performed successively, not collectively, the same procedure as in No. 1 was followed, the organisms used being *M. melitensis*, and cultures derived from skin, breath, and sweat. In none was *M. melitensis* recovered after more than one day's incubation, and in all the mixed broth culture was acid to litmus at the end of each incubation.

Result.—*M. melitensis* incubated in broth in presence of other organisms is recoverable for a very short time, seven days, in presence of alkali producing organisms. One to two days in presence of acid-producing organisms contrasting greatly with its recoverability in pure broth culture, from a tube of which, inoculated December 12, 1904, it was recovered by a sub-culture on agar, April 25, 1905, an interval of over four months.

B. *Vitality of M. melitensis in Pure Culture.*

1. *On agar agar dry.*—Two agar slopes inoculated with *M. melitensis* March 29, 1904, which had been incubated at 37° C. for four days and then placed aside in laboratory cupboard with cotton wool plug unprotected by a rubber cap, had on December 30, 1904, become so dry that no colony could be detached for sub-culture, and the agar itself had contracted to a thin shred; sterile broth was therefore added to the two tubes until the upper level of the dry culture was submerged, these were then placed in the incubator at 37° C. till January 4, when the broth of one had become turbid, this was now sub-cultured, and pure *M. melitensis* was recovered and verified. No growth was obtained from the other.

Two similar slopes inoculated April 5, 1904, examined January 24, 1905, failed to give any growth. Two other such slopes inoculated April 21 and 24, 1904, similarly examined March 20, 1905, failed to give any growth.

Result.—*M. melitensis* had remained alive and capable of reproduction in a dried-up condition on agar from March 29 to December 30 = 276 days (nine months).

2. *In Litmus Milk.*—A tube of litmus milk inoculated with *M. melitensis* (Second Generation, Human Spleen, Bowles), December 12, 1904, yielded *M. melitensis* in sub-culture April 26, 1905, a period of over four months, but in very small quantity, a loopful which in January and February had yielded colonies by the hundred, now giving only 1 to 10 colonies; and after May 5 ceased yielding colonies though experimented with for a fortnight longer, hence, presumably dead after 144 days of vitality.

3. *In Nutrient Ordinary Beef-Peptide Broth.*—A tube of this was inoculated from same source at same time as litmus milk, December 12,

1904, and right up to June 3, 1905, each loopful taken twice weekly for sub-culture was yielding a plentiful supply of *M. melitensis*.

Result.—Still alive and actively reproductive after $5\frac{2}{3}$ months in nutrient broth.

In all these the media were titrated to a reaction of + 10 of acidity with phenol-phthaleine (Eyre's scale).

Results.

| | |
|--|-----------|
| <i>M. melitensis</i> lived on dry agar | 276 days. |
| " " in litmus milk | 144 " |
| " " nutrient broth..... | 173 " |

C. Vitality of M. melitensis in Urine.

A noteworthy feature of the urine of Mediterranean Fever patients is the length of time it remains acid after it has been passed; the following observations given in tabular form demonstrate this. These urines were taken from patients in the wards without special precautions and were kept in the laboratory cupboard, again without any special precautions. The acidity was determined each time by titration against a standard $\frac{N}{5}$ solution of potassium hydrate, phenol-phthallein being used as the indicator, and is expressed according to Eyre's scale. It will be remembered that the optimum reaction of culture media for *M. melitensis* has been found to be an acidity of + 10, Eyre's scale (see Part I of these reports).

| Patient's name. | Date urine passed. | Acidity when passed. | Acidity on Jan. 30. | Acidity on Feb. 21. | Acidity on Mar. 29. |
|-------------------|--------------------|----------------------|---------------------|---------------------|---------------------|
| Anderson | Jan. 3 | + 36 | | + 36 | + 26 |
| Turner..... | 3 | + 5 | | + 2 | - 2 alkaline |
| Martin..... | 3 | + 44 | + 44 | + 40 | + 18 |
| Rentcombe (a) ... | 3 | + 60 | | + 28 | + 4 |
| " | 12 | + 30 | | + 12 | + 12 |
| " (b) | 15 | + 52 | + 40 | | |
| Webb (c)..... | Feb. 5 | + 60 | | | + 50 |
| Jacombe (d) | 5 | + 32 | | | + 32 |

In the following observations on the life of *M. melitensis* in various specimens of urine (again given in tabular form), the following was the method adopted. The specimens of urine were some healthy and some obtained from Malta Fever patients, and one of each sterilised in autoclave at 115°. In each case, after the acidity of the specimen had been determined, 10 c.c. of it were placed in a sterile test-tube with the usual wool plug. As it was also considered of importance to have information as to the number of colonies of other micro-organisms

these specimens contained, each was well shaken and one loopful, taken with a standard loop, distributed over the surface of an agar Petri dish, which was then incubated at 37°, and the number of colonies counted and recorded five days later. After the abstraction of this one loopful, each specimen was inoculated with *M. melitensis*. The same brand was placed in each, a four days' growth on glucose litmus agar of the second generation of *M. melitensis* obtained from the spleen of a fatal case (Bowles) and, as far as possible, the same amount of culture in each case, a small platinum loop being set aside for the purpose of delimiting each time the area of agar to be denuded of growth. The tubes of *M. melitensis* inoculated urine were now placed in the laboratory cupboard (temperature about 15° C.) and daily well shaken and a loopful from each plated on agar, the plate incubated for five days and then examined for *M. melitensis*, which, if found, was verified in the usual way. It was found that after a variable number of days, some morning, a plate from a given urine would contain no *M. melitensis*: this was regarded not as a sign of death of all the *M. melitensis* in the specimen, but as indicating a great diminution in number, and the daily plating persevered with till there had been a succession of seven blank days. Not infrequently a specimen would yield *M. melitensis* one day, then not yield it for one or two days and then again give it. After a succession of seven days' plating without recovery of *M. melitensis*, that particular observation was terminated with the then acidity of that particular specimen being determined and recorded.

The following were the results obtained :—

| Source of urine used. | Reaction. | No. of colonies per loop. | No. of days <i>M. melitensis</i> was recovered. | Reaction of urine at end of observation. |
|------------------------------------|-----------|---------------------------|---|--|
| Unsterilised, normal healthy ... | + 7 acid | 57 | 2 | Very alkaline. |
| " " " ... | + 8 " | 35 | 5 | Just neutral. |
| " " " ... | + 7 " | 57 | 2 | Alkaline, strongly. |
| " " " ... | + 8 " | 5 | 33 | - 5 alkaline. |
| Unsterilised, Malta Fever patient | + 18 " | 13 | 15 | - 40 " |
| (a) " " " " | + 60 " | 32 | 24 | - 25 " |
| (b) " " " " | + 52 " | 7 | 18 | - 25 " |
| " " " " | + 40 " | 6 | 43 | Just neutral. |
| (c) " " " " | + 60 " | 175 | 36 | + 16 acid. |
| (d) " " " " | + 32 " | 51 | 49 | + 4 " |
| Sterilised, normal healthy..... | + 8 " | Nil | 17 | + 2 " |
| Sterilised, Malta Fever patient... | + 40 " | " | 33 | + 20 " |

A control inoculation of the same culture of *M. melitensis* into nutrient broth, kept under same conditions, was recovered by sub-culture on to agar after 4½ months.

Remarks.—Here neither differences in acidity or in number of other organisms seem to have had an appreciable influence on the duration of life of *M. melitensis* in urine, variations in this being presumably due to variations in other constituents so far as urine derived from Malta Fever patients is concerned, though there does seem to exist a direct connection between duration of life of *M. melitensis* and number of other organisms in the case of normal healthy urine, the greater the number of the latter the shorter the life of *M. melitensis* in such urine containing both. The urines lettered (a), (b), (c) and (d) in the two Tables I and II were identical, and the date of last determination of acidity in Table I was after the final one in Table II. The factor of difference was the presence of *M. melitensis* in urines of Table II and its absence in those of Table I, and I think the development of alkalinity in the urines of Table II compared with its non-development in the identical urines of Table I is attributable to the presence of *M. melitensis* in those of Table II, this being in accord with other observations on production of alkalinity by *M. melitensis* in nutrient media (see Part I of these reports).

The salient feature, however, is the comparatively long retention of reproductive activity of *M. melitensis*, lasting as long as seven weeks, in the urine of Mediterranean Fever cases. I may remark here that I find that 23 out of 30 samples of urine from Mediterranean Fever cases examined effected agglutination of *M. melitensis* in varying degrees; evidently agglutinins are excreted in the urine.

D. *Vitality of M. melitensis in Diluted Urine.*

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. of *fresh healthy urine*, which was well shaken and then added to 100 c.c. of sterilised tap water contained in a flask with cotton wool plug, the idea being to simulate the diluted fluid of the ordinary urinal minus accessory contaminations. Here again the flask was daily well shaken and a loopful plated on agar, the plate incubated and examined for *M. melitensis* colonies in the usual way. *M. melitensis* was recovered for nine days.

2. The same experiment was repeated, using *urine* from a *Malta Fever* case, and *M. melitensis* was recovered day by day, with occasional intervals of one, two, or three days, for 79 days; the daily sub-culture was persevered with for 14 days longer without *M. melitensis* being recovered.

Result.—1. *M. melitensis* was recovered from diluted healthy urine for nine days.

2. *M. melitensis* was recovered from diluted Mediterranean Fever urine for 79 days.

E. Vitality of M. melitensis in Urine—Contaminated Milk.

1. The same brand and quantity of *M. melitensis* was placed in 1 c.c. fresh healthy urine, which was well shaken and then added to 100 c.c. of sterilised goat's milk contained in a wool-stoppered flask, which was thoroughly well shaken every morning, and then a loopful plated on agar, incubated and examined for *M. melitensis* colonies. *M. melitensis* was recovered for three days, but after that was completely crowded out by other colonies.

2. The same experiment was repeated, using *Malta Fever* urine instead of healthy urine. In this case *M. melitensis* was recovered for 38 days.

Result.—1. *M. melitensis* recovered from milk contaminated with healthy urine for three days.

2. *M. melitensis* recovered from milk contaminated with *Mediterranean Fever* urine for 38 days.

F. Vitality of M. melitensis in Urine Dried on Fabrics.

The intention was here to obtain information as to the possible infectivity of garments soiled with urine containing *M. melitensis*.

1. Ten c.c. of normal healthy urine were taken and inoculated with the same brand and quantity of *M. melitensis* as in the preceding urine experiments, pieces of sterile lint were immersed in it till saturated, then removed and allowed to dry in a sterile Petri dish at the laboratory temperature (about 15° C.), which took four days. Then daily two small pieces were snipped off with sterile scissors, one put in a 10 c.c. broth tube, the other used to make impressions on the surface of agar in a Petri dish, by lifting it from area to area of the agar with a pair of forceps, and in each new situation pressing it on to the surface of the agar with a platinum spreader. The broth-tube and plate were then incubated and examined for *M. melitensis* in the usual way, but in this experiment none were recovered.

2. Precisely the same experiment, but using navy blue serge No. 3, such as is worn by the bluejacket. Again the result was the same, no *M. melitensis* was recovered.

3. Thinking that the failure to recover *M. melitensis* in the two preceding experiments might be due to the very slow drying on the fabric and the consequent facility for fermentation of the urine which certainly took place, the same experiments were repeated, using *Malta Fever* urine and drying the fabrics in the incubator at 37° C.; this was found to take only 24 hours instead of the four days requisite at atmospheric temperature.

Result.—*M. melitensis* was recovered from the lint so treated for five days, and from the blue navy serge for 78 days; daily sub-inoculations having been made as described in F 1. The difference between the

periods of recovery in the two cases may doubtless be attributable to the very different modes of manufacture of the two fabrics.

G. *Vitality of M. melitensis in Sterilised Tap Water.*

Ten c.c. of ordinary tap water were taken in a test-tube with cotton wool plug and sterilised in autoclave at 115° and then inoculated with same brand and quantity of *M. melitensis* as in the urine experiments, C 2, and placed in laboratory cupboard at temperature of about 15° C. Each day this tube was well shaken and cultured as described in C, by means of standard platinum loop and spreader on agar in Petri dishes.

Result.—*M. melitensis* was recovered for 50 days.

H. *Vitality of M. melitensis in Unsterile Tap Water.*

The same experiment as G, but the tap water not sterilised and two observations were made.

1. Commenced December 12, finished December 29. *M. melitensis* recovered for 10 days, tap water from rain tank on roof being used; this is not usually considered potable.

2. Commenced December 30, finished March 23. *M. melitensis* was recovered for 72 days, tap water from ordinary urban house-supply being used, which is used for drinking purposes.

Result.—1. *M. melitensis* was recovered from tank water for 10 days.

2. *M. melitensis* was recovered from potable water for 72 days.

I. *Vitality of M. melitensis in Unsterile Sea Water.*

This experiment was made twice in the same way as those described under G and H, the same brand and quantity of *M. melitensis* being used. The sea water was obtained from the area of the Grand Harbour in which H.M.S. "Egmont" (a stationary dépôt ship on board of which a varying number of 300 to 600 men are living) is moored, and in close proximity to this ship, with the sewage of which it was demonstrably fouled.

Result.—1. The first specimen of sea water yielded three colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 46 days.

2. The second specimen of sea water yielded 11 colonies per loop of other micro-organisms before inoculation, and *M. melitensis* was recovered from it after inoculation for 11 days.

J. *Vitality of M. melitensis Dry on Cover Slips.*

A large number of cover slips were cleaned and sterilised, an emulsion of same brand of *M. melitensis* as that used in all the preceding experiments made in sterilised distilled water, and one drop

of this placed with standard platinum loop on one surface of each cover slip, these being arranged in rows inside sterile Petri dishes wherein the drops of emulsion were allowed to dry, the whole being kept in laboratory cupboard at about 15° C. Each day one was removed with a pair of sterile forceps and placed, *M. melitensis* film-side downwards, on the surface of agar contained in a Petri dish, over which it was moved by the forceps till all the *M. melitensis* film had apparently been left distributed over the agar surface, when it was left *in situ*, still with its "film" side adhering to the agar, the cover of the Petri replaced numbered and dated, and the whole incubated for five days and then examined for growth.

Result.—*M. melitensis* was recovered in this way from these cover slips for 15 days; a result of some importance as showing the inherent vitality of *M. melitensis* even when dried and separated from any trace of organic matter. It will be remembered that in the dry condition on organic matter (nutrient agar) it lived for over nine months (*vide* Section B of these experiments).

K. *Vitality of M. melitensis in Earth.*

1. *In Sand Free from Organic Matter.*—The sand used was a silicious red sand obtained from North Africa; it was heated to redness to burn off organic matter, then well shaken with distilled water, the reaction of which was after this found to be neutral. Some of it was then sterilised in dry air at 160° C. inside a pair of watch glasses held in a clip. An emulsion of the same quantity and brand of *M. melitensis* as in preceding experiments (Second Generation from Human Spleen, Bowles) made in 5 c.c. of distilled sterilised water and well mixed with the sterilised sand, and the whole allowed to dry in a cupboard at the temperature of the laboratory, 15° C. Twice a week two specimens were put out to incubate as follows: (a) A little of the inoculated sand was put in a 10 c.c. broth tube which was then incubated for five days, after which a loopful was put out on a glucose-litmus-agar slope which was incubated and examined for growth; (b) A little was put on the surface of similar agar in a Petri dish, sufficient nutrient broth added to make a mud of it, and this was then spread out with a Klein's platinum spreader, and, after five days' incubation at 37° C., examined for growth. I found that this method gave just as constant results as the former (a), and it had the advantage of saving one incubation and the corresponding number of days in time.

Result.—*M. melitensis* was recovered in this manner for 16 days.

Remark.—This experiment and the last are quite comparable in that the *M. melitensis* culture used was not only the same, but that it was kept dry with an inorganic environment free from organic matter, and

the duration of reproductive vitality was much the same; on cover slips 15 days, in sand 16 days.

2. *In Various Malta Soils.*—In preliminary experimentation on the sterilisation of these, they were all found after sterilisation by dry heat to be excessively alkaline, a condition seriously prejudicial to the vitality of *M. melitensis*. This was found to be due to the large amount of calcium carbonate they contained, some of which, by the dry heat used, was converted into calcium oxide, which on the addition of water became calcium hydrate. This caused one to examine the reaction of the various soils as received, by thoroughly stirring up and shaking each, and then shaking a little in distilled water in a test-tube and taking the reaction of that. All the specimens were thus found to be slightly alkaline to begin with. So sterilisation was now done by putting the specimen of soil in a beaker, half filling this with distilled water and placing it in the autoclave at 115° for 30 minutes; this was found to effect sterilisation without any alteration of alkalinity.

(a) *In Greyish Yellow Soil, with additional Organic Matter.*

This soil was personally obtained from a field in Sliema, was sterilised as just described, sterility verified by broth culture, a portion in bulk equal to about 10 c.c. placed in a sterile test-tube and put in incubator at 37° C. till dry, then on to it was poured, by means of a sterile pipette, a broth growth of *M. melitensis* (still Second Generation from Human Spleen, Bowles), till its upper $\frac{1}{3}$ was quite saturated with moisture, then tube was plugged with sterile wool and placed in laboratory cupboard. Twice in each week two portions from the upper surface were planted out in the manner described in K 1 for sand, incubated, and examined for growth. It was noticed, as the experiment progressed, that the upper surface of the soil got apparently dry, but that at the bottom of the test-tube it became damp from percolation of the nutrient broth downwards, and this dampness persisted till the conclusion of the experiment; so that the *M. melitensis* present must have been continually in the presence of water vapour. As in K 1, it was found that planting out on agar, as described, gave just as constant results as planting out in broth and then sub-culturing from this.

Result.—*M. melitensis* was thus recovered from this soil for 91 days.

(b) *In Reddish Soil without additional Organic Matter.*

This soil was also personally obtained from a Sliema field; it was very similar in composition to that used in the preceding experiment, differing mainly by containing a little under 1 per cent. of iron oxide, to which its reddish colour was due. It was sterilised under water in the autoclave at 115° C., sterility verified by broth culture, and sufficient placed in a small sterile Petri dish (3 cm. in diameter) to give a depth of about $\frac{3}{16}$ to $\frac{1}{4}$

of an inch. An emulsion of same quantity and brand of *M. melitensis* as in Experiment K 1 was made in 5 c.c. of distilled sterilised water, and, by means of a sterile pipette, was distributed all over the surface of the sterilised soil contained in the Petri dish. Two small portions of this were planted out in agar and in broth twice weekly in the manner already described, incubated and examined for growth.

Result.—*M. melitensis* was thus recovered from this soil for 80 days.

(c) *In White Soil without additional Organic Matter.*

This specimen of soil was obtained from an area of land where building operations were going on, and consisted very largely of the *débris* from the stone cutting, shaping, and smoothing operations which, as is usual in Malta, were carried out on the spot where the finished stone was wanted for use. It is a very soft friable Globigerina limestone, and the *débris* used contained only a mere trace of organic matter. With this the experiment just described was exactly repeated in every particular.

Result.—*M. melitensis* was recovered for 24 days.

(d) *In Recently well-manured Soil, Sterilised and kept Wet.*

This experiment was intended to contrast with the latter in the amount of organic matter present in the soil, it being very great in this experiment, very little indeed in the last one, and also in the amount of water present, a similar difference being maintained. Recently (five weeks) manured soil was obtained from the Argotti Botanical Gardens, dried, pulverised in a mortar, sterilised as in the preceding three experiments, an amount equal in bulk to about 10 c.c. put in a sterile test-tube and well shaken down; and then on to it was poured, by means of a sterile pipette, an emulsion of *M. melitensis* grown on agar made from the same quantity, brand, and generation of *M. melitensis* as in the preceding experiments, in 5 c.c. of distilled sterilised water. This soil was further kept saturated with moisture by dropping on it from time to time sterilised tap water from a sterile pipette, and was kept at laboratory temperature (15° C.) for the whole period of the experiment. From it two portions were planted out in broth, and on agar twice weekly in the way described in K 1, then incubated and examined for growth. The experiment was started on December 14, 1904, and each planting out yielded *M. melitensis*, which, as usual, was duly verified. During the examination and verification of the growth of January 8, it was noticed that the resulting colonies, while resembling the usual *M. melitensis* colony in every other particular, had less sharply defined, less abrupt margins, and that the microscopical preparations contained a few bacillary forms. By February 12 the new colonies of that date presented slightly crenated edges, shading away on the agar, though quite similar in size and shape

to standard *M. melitensis* colonies of same duration of growth on same agar, and now consisted almost entirely under the microscope of small bacillary forms, when stained, of about $\frac{2}{3}$ the diameter of a normal *M. melitensis*, and three times its length. A sub-culture of this was now put through all the tests specified in Part I of these Reports, for the recognition and verification of *M. melitensis*, behaving in all particulars like standard *M. melitensis* save in the morphological details mentioned. These bacilli in hanging-drop preparations were feebly motile. Many specimens were stained for flagellæ according to Rossi's method, but none were demonstrated. It will be remembered that Gordon, in a paper in the *Lancet*, March 11, 1899, described flagellæ in connection with *M. melitensis*. I have not succeeded in verifying this. Successive sub-cultures of this growth of February 12, for 10 generations, in broth and on agar were now made, but there was no reversion to the coccal form; the Tenth Generation was exactly like the First; but a sub-culture from the Ninth Generation into peptone water (made for the purpose of ascertaining the presence or absence of indol and nitrite formation, neither present), showed in a stained specimen both cocci and bacilli, and intermediary forms such as French bacteriologists speak of as "cocco-bacille." On February 22 a rabbit which had never been experimented upon, and whose blood gave no trace of agglutination with standard *M. melitensis*, was injected intra-cerebrally with the usual aseptic precaution with $\frac{1}{2}$ c.c. of an emulsion made from this same Ninth Generation. Its temperature rose to 105° F. the same evening and 106° F. the following evening, after which it fell to normal and never rose again. On February 25 there was a distinct agglutination reaction on *M. melitensis*, with its blood serum in a dilution of $\frac{1}{32}$; this had increased on March 5 to a dilution of $\frac{1}{300}$. On February 28 1 c.c. of blood was taken aseptically from the animal's left internal saphenous vein, placed in 19 c.c. of nutrient broth, and incubated in the usual way. There being absolutely no trace of growth obtainable from this up to March 10, the animal, which had fully recovered from the operation, was that day chloroformed, a *post-mortem* made, and inoculations made into both broth and agar from the brain, heart's blood, urine, spleen, liver, and kidneys; no growth was obtained from any of these.

Concurrently with all this, the periodical plantings out had been carried on, but no growth was obtained from any planting out later than March 7, though these were continued till March 22.

Remarks.—A bacillary form in pure cultures of *M. melitensis* has been noticed by various workers, but not apparently in so marked a degree as in this instance. As an intra-cerebral injection of the coccal form of *M. melitensis* usually produces in a rabbit death in four or five days with presence of *M. melitensis* in the various organs, the bacillary form produced, as described, is obviously of less virulence.

Result.—*M. melitensis* was recovered from this sterilised, manured, and saturated soil rich in organic matter for 83 days, a bacillary form of *M. melitensis* deficient in virulence being developed.

(e) *In Recently Manured Non-Sterilised Soil.*

The same soil as in the last experiment was used and treated in precisely the same way save that it was not sterilised.

As it was anticipated that very rapid overgrowing of the *M. melitensis* put in (the viability of which was as usual tested by a control) would take place, a little was planted out daily, a small portion being placed on the surface of agar in a Petri dish, made into mud with nutrient broth, and distributed by means of a Klein's platinum spreader all over the surface of agar in three successive Petri plates. Although in this manner discrete isolated colonies were obtained in the third plate, no *M. melitensis* was ever recovered, though four separate repetitions of the experiment were made.

Result.—*M. melitensis* speedily crowded out by the other organisms present.

Summary of Results obtained as to Vitality of M. Melitensis Outside the Body, the same Brand, Generation and Quantity of M. melitensis being used throughout (except in B 1).

| | Days. |
|--|-----------------------------|
| A. In mixed broth culture with— | |
| 1. Laboratory contamination..... | 7 |
| 2. " " | 7 |
| 3. Organisms derived from sweat, skin and urine | 2 |
| 4. " " " " | Nil |
| 5 to 10. " " " " and breath ; in each | 1 |
| B. In pure culture media with a reaction of + 10 acid— | |
| 1. On agar slope (source of <i>M. melitensis</i> not noted) | 276 |
| 2. In litmus milk (source of <i>M. melitensis</i> as in all other experiments) | 144 |
| 3. In peptone broth (ditto) | 173 |
| C. In urine (persistent acidity of Mediterranean Fever urine as described)— | |
| 1. In unsterilised normal healthy urine, four experiments | 2, 5, 2 and 33 |
| 2. " " Malta Fever urine, six experiments | 15, 24, 18 43, 36 and 49 |
| 3. " sterilised normal healthy urine, one experiment ... | 17 |
| 4. " " Malta Fever urine, one experiment .. | 33 |

| | Days. |
|---|---------------|
| D. In diluted urine— | |
| 1. Healthy urine diluted 100 times with sterile tap water | 9 |
| 2. Mediterranean Fever (ditto) | 79 |
| E. In urine contaminated milk— | |
| 1. Goat's milk contaminated with 1 per cent. healthy urine | 3 |
| 2. " " 1 " Mediterranean Fever urine | 38 |
| F. In urine dried on fabrics— | |
| 1. In Mediterranean Fever urine dried on lint | 5 |
| 2. " " " navy serge ... | 78 |
| G. In sterilised tap water | 50 |
| H. In unsterilised tap water— | |
| 1. Tank water | 10 |
| 2. Potable water | 72 |
| I. In unsterile sea water, two experiments | 11 and 46 |
| J. Dry on cover slips | 15 |
| K. In various earths— | |
| 1. In sand free from organic matter | 16 |
| 2. " various Malta soils— | |
| a. In sterilised grey-yellow soil with added organic matter..... | 91 |
| b. In sterilised reddish soil without added organic matter..... | 80 |
| c. In sterilised white soil almost free from organic matter..... | 24 |
| d. In sterilised well-manured soil rich in organic matter (bacillary forms of <i>M. melitensis</i> developed) | 83 |
| e. In non-sterilised well-manured soil | Not recovered |

IV. ON THE RECOVERY OF *MICROCOCCUS MELITENSIS* FROM THE URINE OF MEDITERRANEAN FEVER PATIENTS.

By J. CRAWFORD KENNEDY, Captain R.A.M.C., Member Mediterranean Fever Commission, Malta, April, 1905.

Since September, 1904, this work has been more than trebled, and special attention has been paid to the quantities excreted and to the period of disease during which the excretion is greatest.

The following table is a summary of the work done :—

| | No. of samples examined. | No. of times <i>M. melitensis</i> recovered. |
|----------------------|-----------------------------|--|
| September | 347 | 6 |
| October | 217 | 6 |
| November | 581 | 63 |
| December | 398 | 43 |
| January | 201 | 19 |
| February..... | 110 | 19 |
| March to April 2 ... | 120 | 30 |
| Total | 1974 | 186 |

Percentage of recoveries..... 9½ per cent.

The number of cases examined was 61, and from 33 of these *M. melitensis* was recovered. Therefore *M. melitensis* was recovered from 54 per cent. of the cases examined. Deduct from this the cases which were examined less than 10 times—it leaves 50 cases and 31 recoveries, or 62 per cent. ; 43 cases were examined over 20 times, with 31 recoveries, or 72 per cent.

The method of examination was the same as described by Major Horrocks in a former report, and each recovery put through the usual tests.

In order that the work may be taken in at a glance, I have prepared a list of the cases from which *M. melitensis* was recovered, giving particulars of number of samples and recoveries, quantity and period of disease, also a chart of the temperature. In those cases which supplied many recoveries the whole chart is given, in those with only one recovery, only the previous and subsequent two or three days' temperature is given.

List of Cases from which *M. melitensis* has been Recovered, giving particulars of Quantity and Period of Disease.

| No. | Name. | No. of samples examined. | No. of times <i>M. melitensis</i> recovered. | Greatest No. of colonies <i>M. melitensis</i> found in a cubic centimetre urine. | No. of times <i>M. melitensis</i> recovered when temperature of previous or of subsequent 24 hours not above 99°. | Earliest and latest day of illness on which <i>M. melitensis</i> recovered. | Remarks. |
|-----|----------------------|--------------------------|--|--|---|---|--|
| 1 | Kinsella | 97 | 27 | 189 | 16 | 21 111 | See chart. This case examined all through illness. The excretion of <i>M. melitensis</i> was almost entirely during convalescence. |
| 2 | Bean | 44 | 23 | 450 | 7 | 58 84 | See chart. A normal temperature for 7 days during excretion of <i>M. melitensis</i> . |
| 3 | Ralph | 76 | 18 | 440 | 2 | 39 133 | See chart. Excretion of <i>M. melitensis</i> after 33 days' normal temperature. |
| 4 | Smith (Rifles) | 64 | 6 | 309 | — | 74 114 | See chart. Excretion after 27 days of practically normal temperature. |
| 5 | Gane | 29 | 14 | 1068 | 7 | 108 145 | See chart. Excretion after 30 days' normal temperature. |
| 6 | Charlton | 35 | 4 | 18 | 4 | 82 102 | See chart. Colonies so thick in one sample as to be uncountable—from 500 to 800 in one drop of urine. |
| 7 | Bolt | 151 | 23 | 129 | — | 74 165 | See chart. Another case of a sudden gush of <i>M. melitensis</i> in urine. |
| 8 | Anthony | 91 | 3 | Innumerable | — | 81 156 | See chart. Excretion after 12 days' normal temperature. |
| 9 | Surmin | 93 (74 days) | 48 (41 days) | Innumerable | 27 | 174 249 | See chart. Excretion after 15 days' normal temperature. |
| 10 | Groom | 70 | 1 | 3 | 1 | 82 | |
| 11 | Mitchell | 4 | 1 | 6 | 1 | 77 | |
| 12 | Rivers | 4 | 1 | 3 | 1 | 43 | |
| 13 | Cannole | 31 | 2 | 3 | 2 | 102 112 | |

List of Cases from which *M. melitensis* has been Recovered, giving particulars of Quantity and Period of Disease—*contd.*

| No. | Name. | No. of samples examined. | No. of times <i>M. melitensis</i> recovered. | Greatest No. of colonies of <i>M. melitensis</i> found in a cubic centimetre urine. | No. of times <i>M. melitensis</i> recovered when temperature of previous or of subsequent 24 hours not above 99°. | Earliest and latest day of illness on which <i>M. melitensis</i> recovered. | Remarks. |
|-------------|----------------|--------------------------|--|---|---|---|--|
| 14 | Campbell | 71 | 2 | 6 | — | 93 | See chart. |
| 15 | Walker | 25 | 2 | 116 | — | 55 | See chart. |
| 16 | Silburn | 23 | 2 | 9 | 2 | 60 | See chart. Excretion during convalescence. |
| 17 | Turner | 80 | 1 | 3 | — | 52 | See chart. |
| 18 | Bagwell | 29 | 1 | 3 | — | 77 | See chart. |
| 19 | Rentcome | 40 | 1 | 4 | 1 | 99 | See chart. |
| 20 | Marchant | 55 | 1 | 15 | — | 72 | See chart. |
| 21 | Donovan | 46 | 1 | 3 | 1 | 57 | See chart. |
| 22 | Bennett | 41 | 1 | 4 | 1 | 83 | Excretion after 24 days' normal temperature; quite convalescent. |
| Total | | 1199 | 183 | Earliest day recovered | | 21 | Out of 20 cases examined during convalescence 11 were found to be excreting <i>M. melitensis</i> . |
| Average... | | — | 15·2 per cent. | Latest day recovered | | 249 | |

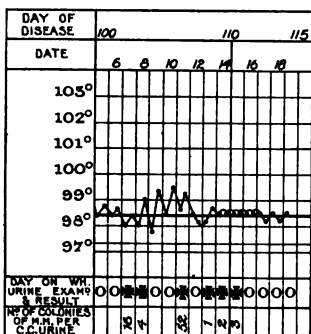
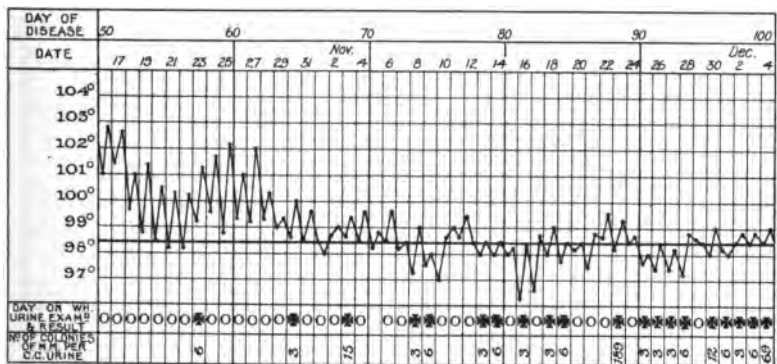
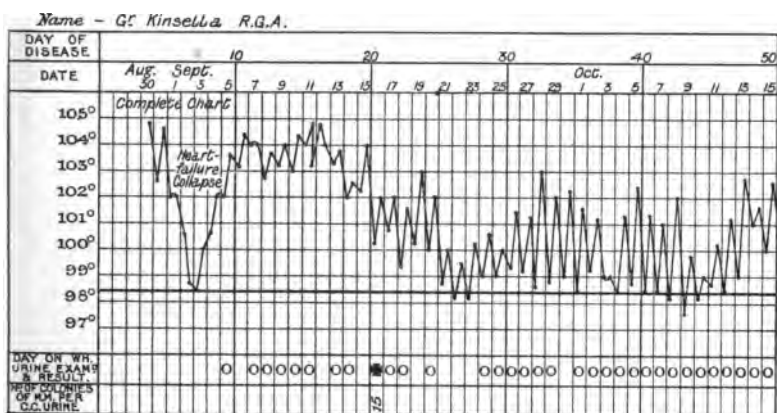


Chart 1.—KINSELLA.

Name - Pte. Bean Rifle Bde.

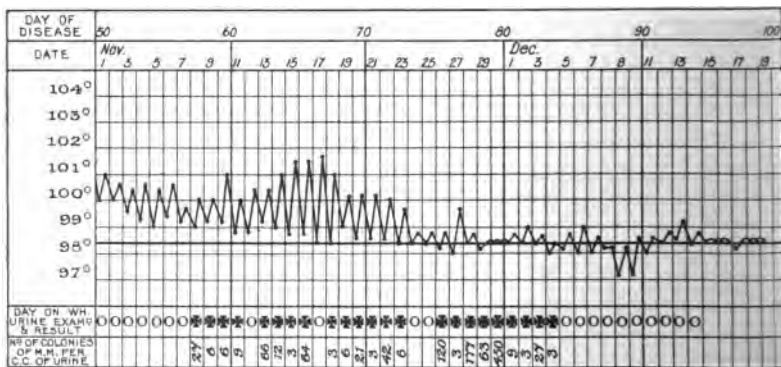
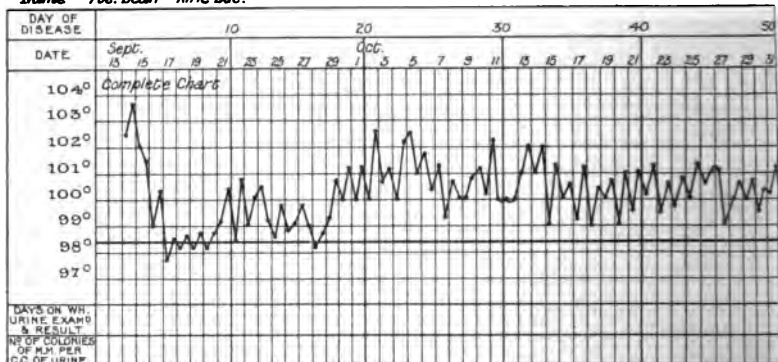


Chart 2.—BEAN.

Name - Pte. Ralph 4638 R.W.Kent.

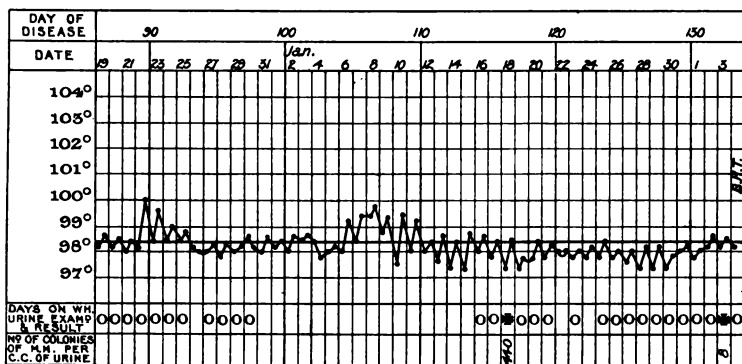
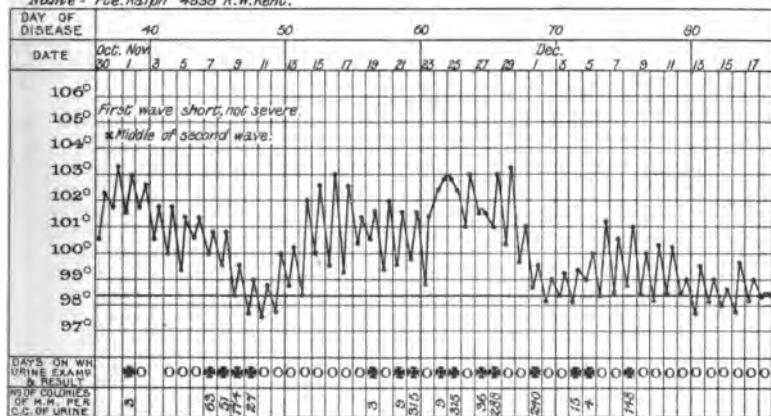


Chart 3.—RALPH.

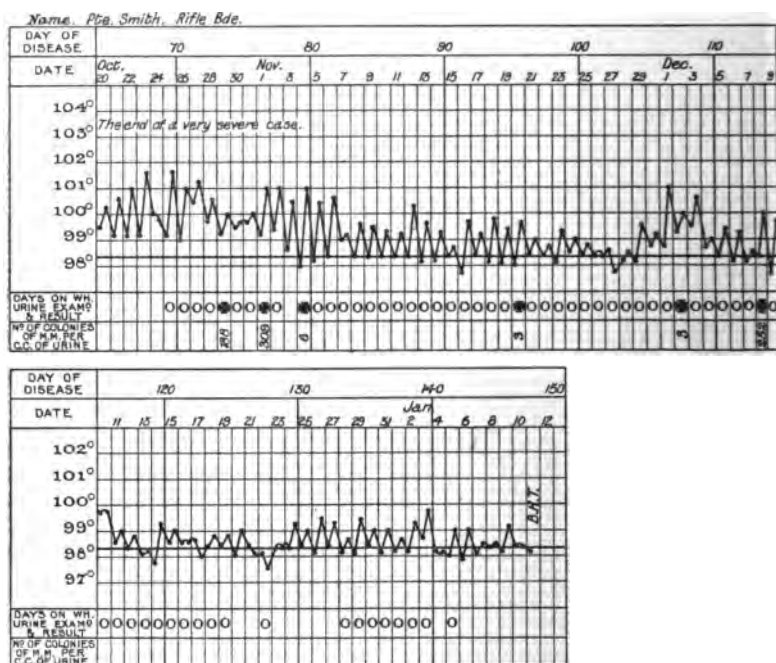


Chart 4.—SMITH.

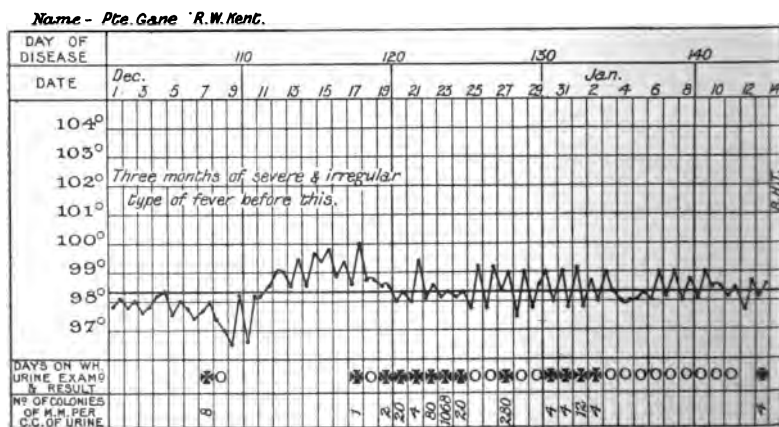


Chart 5.—GANE.

Name - VCpl. Charlton 'Rifle Bde.

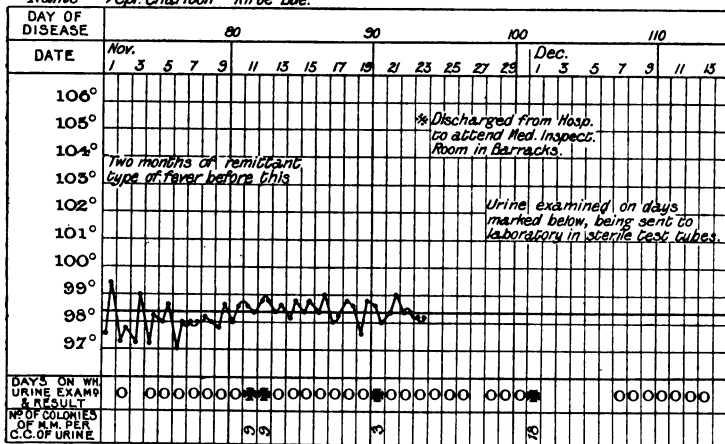


Chart 6.—CHARLTON.

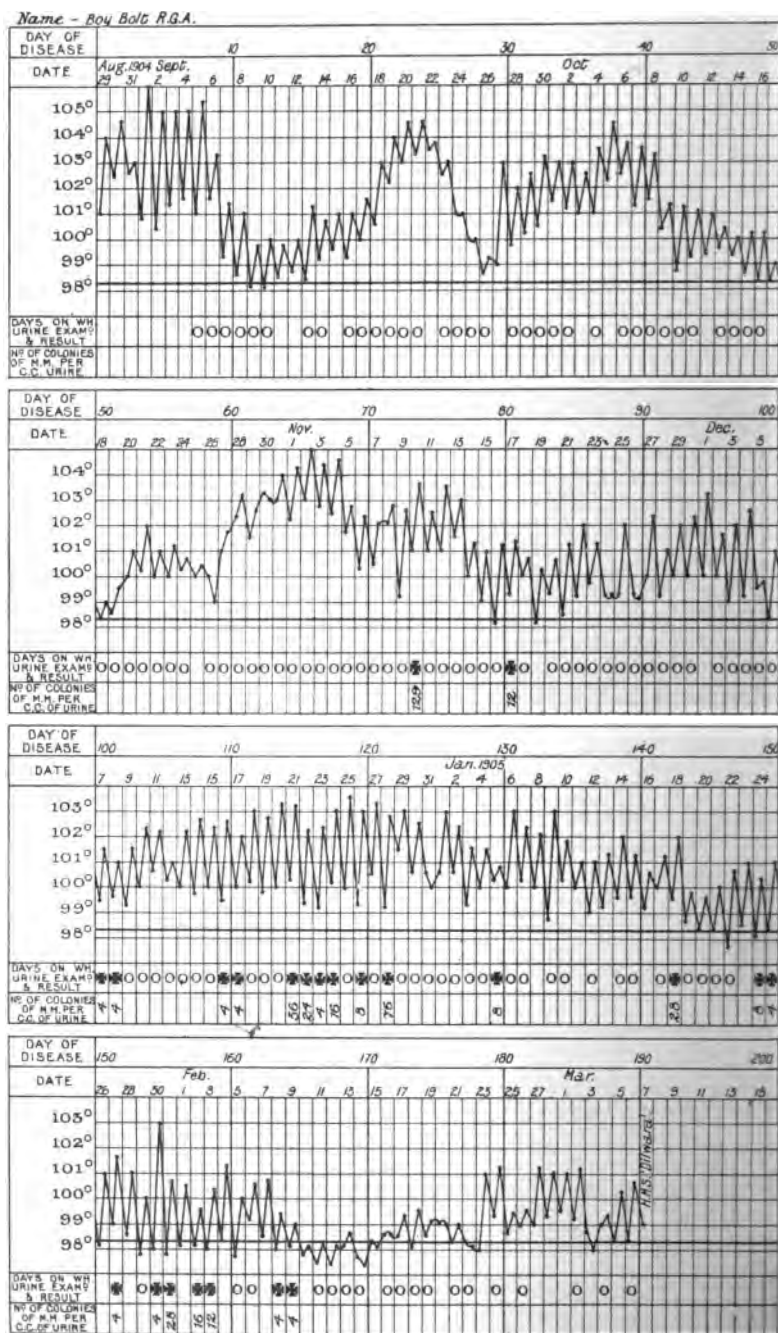
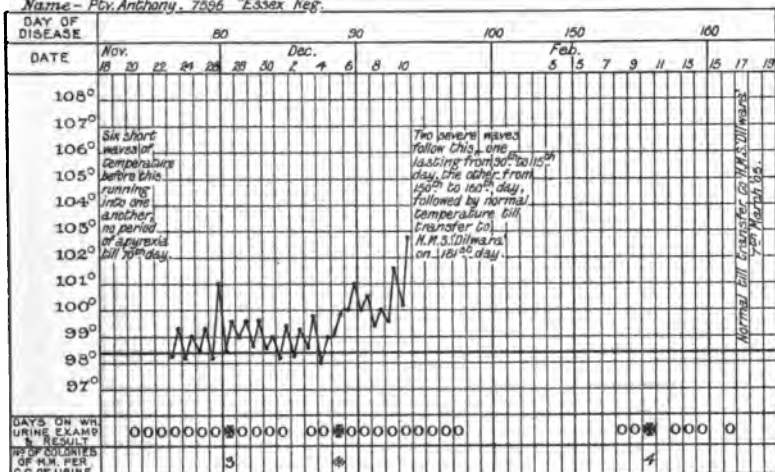


Chart 7.— Bolt.

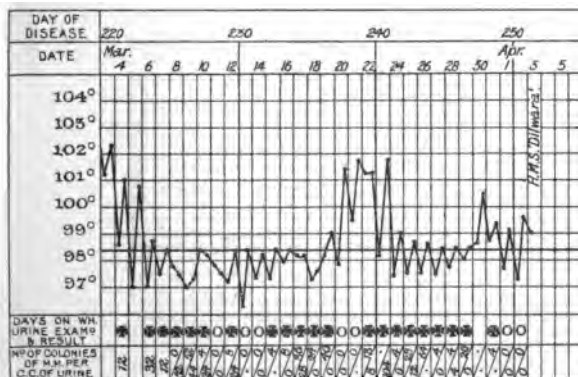
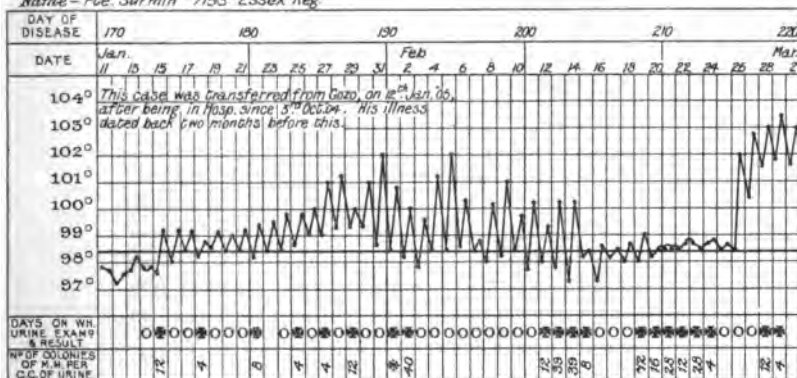
Name - Ptv. Anthony. 7596 ²Essex Reg.



* Colonies absolutely innumerable, any number between 500 to 800 in each drop of urine.

Chart 8.—ANTHONY.

Name - Pte. Surmin 7153² Essex Reg.



◆ Colonies on plate were innumerable. Almost as thick as an artificial emulsion.

Chart 9.—SERMIN.

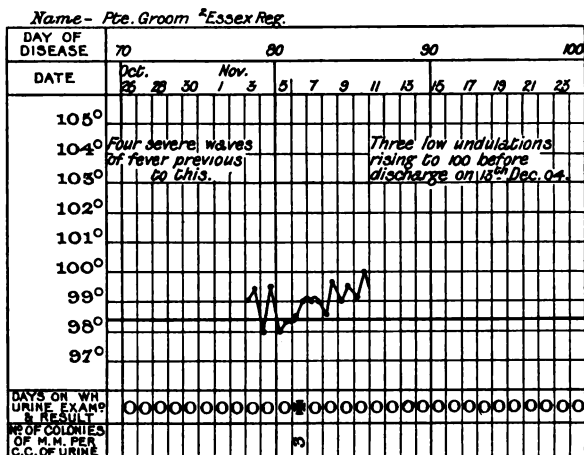


Chart 10.—GROOM.

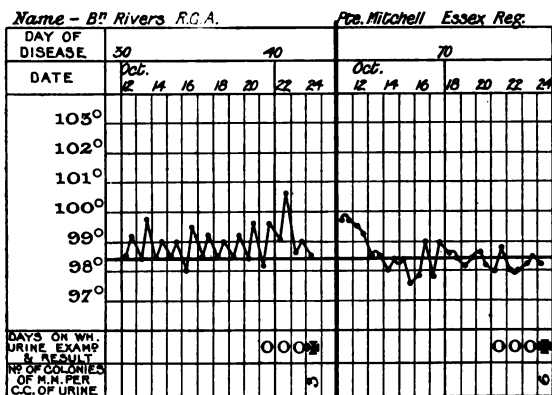


Chart 12—RIVERS.

Chart 11.—MITCHELL.

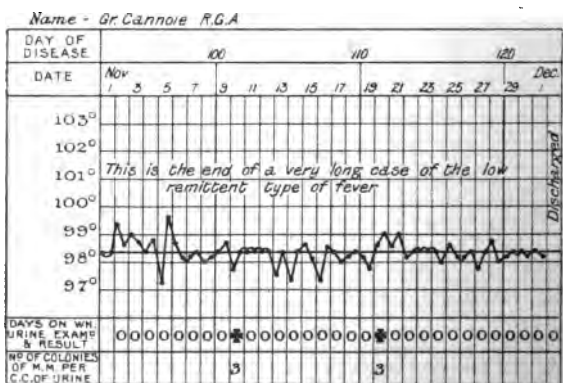


Chart 13.—CANNOIE.

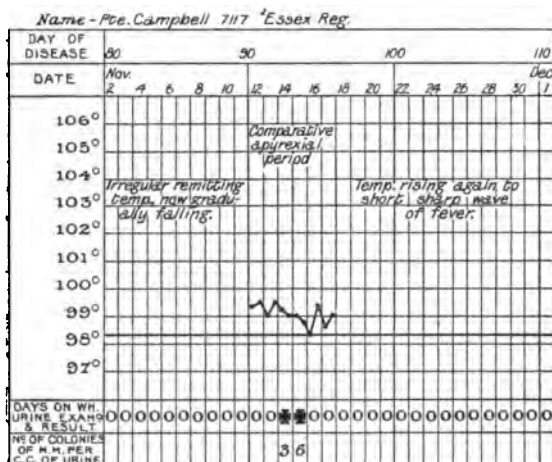


Chart 14.—CAMPBELL.

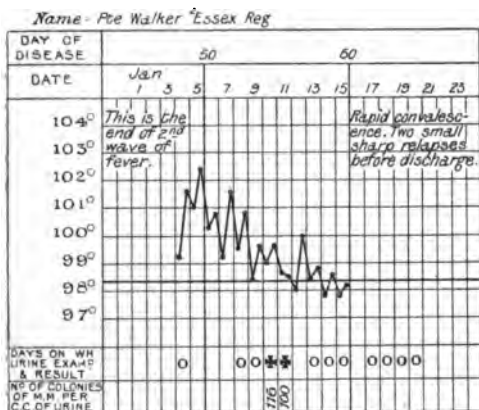


Chart 15.—WALKER.

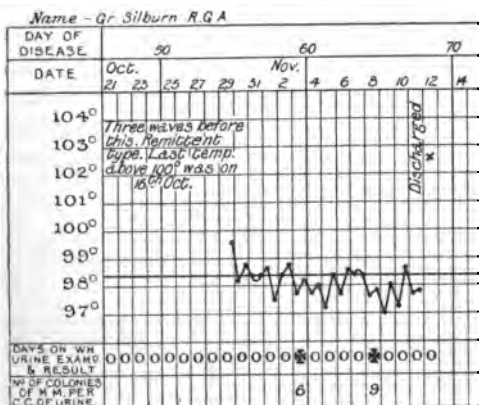


Chart 16.—SILBURN.

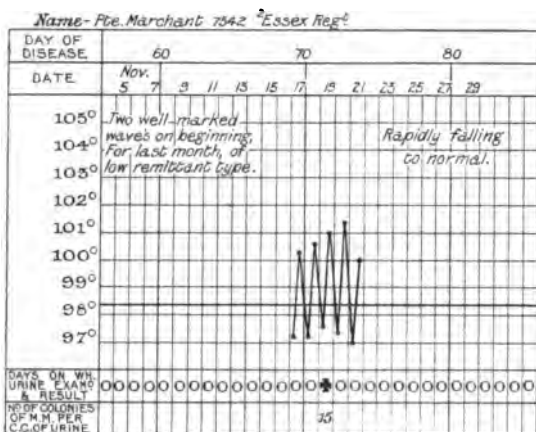


Chart 20.—MARCHANT.

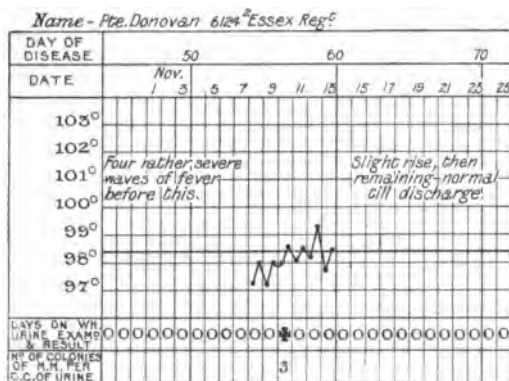


Chart 21.—DONOVAN.

Summary.—The excretion of *M. melitensis* in the urine would appear to be of two kinds :—

- (1) A sudden enormous gush which stops as suddenly as it appears.
- (2) A long continued excretion of small quantities.

As examples of the first see Cases Nos. 8 and 9.

As examples of the second see Cases Nos. 1, 2, 5, 7 and 9.

The period of disease most favoured is early convalescence or the last stages of the fever, especially just as a "wave" of fever is subsiding and the temperature reaching normal. It will be noticed that excretion tends to stop if another "wave" begins (see Charts 3, 8 and 9).

The time of day or rather the period of the 24 hours during which

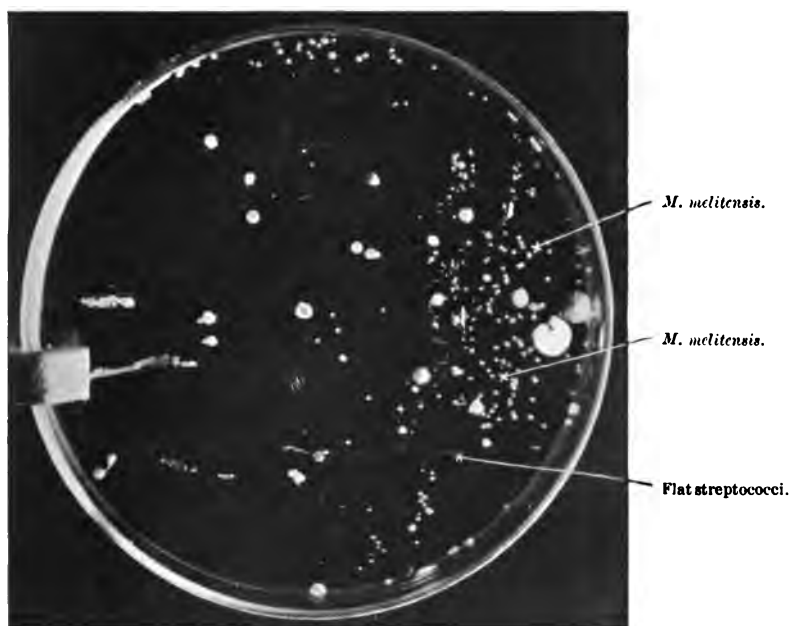
the excretion is greatest, appears to be between 6 P.M. and 6 A.M. Case No. 9 was for some time examined morning and evening, viz., the urine passed at 6 A.M. and that passed at 6 P.M. On 14 days on which the urine was examined at both these times *M. melitensis* was recovered, and taking the average daily excretion for the morning and evening it was found to be 20 colonies per cubic centimetre for the morning to 14 for the evening. On the 14 days mentioned above, *M. melitensis* was found 13 times in the morning urine and only eight times in the evening (see Chart).

The length of time after recovery during which the excretion may continue appears to be considerable, see Cases Nos. 3, 5 and 6. In the case of No. 6, the patient was discharged from hospital cured, but with orders to attend daily at the Medical Inspection room. I supplied him with sterile test-tubes and he daily supplied me with a fresh sample of his morning urine; on the seventh day after his discharge I found six colonies per $\frac{1}{3}$ c.c.

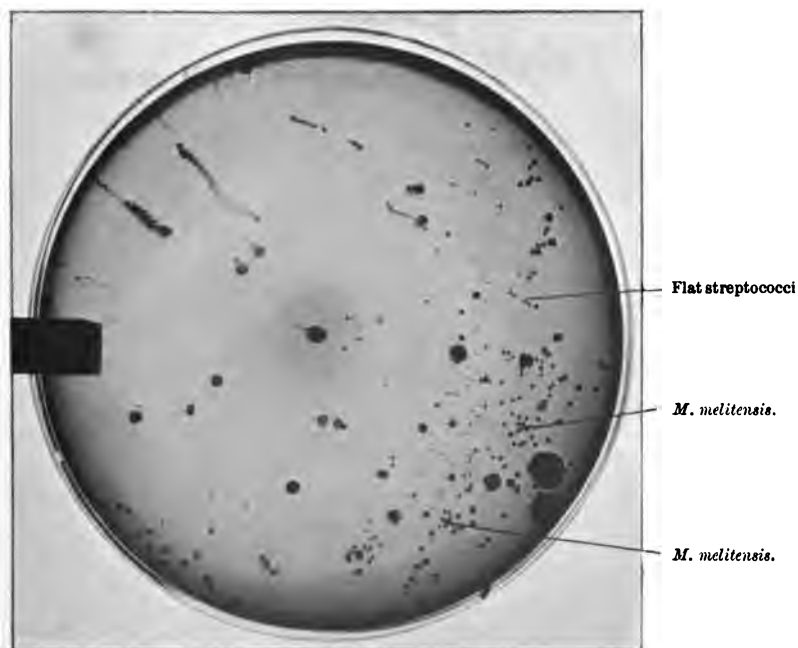
The longest period (temperature not above 99) after which excretion has been proved is 33 days; but see Case No. 1, where from the 66th day to the 111th day temperature never reached 100, yet *M. melitensis* was recovered all this time. There were, however, slight periodical rises to 99°·4, indicating the presence of infection in the system.

The most severe and prolonged cases were the ones that provided the most recoveries. The latest day of disease on which *M. melitensis* was recovered was the 249th.

I attach the photographs of a plate made by planting out $\frac{1}{4}$ c.c. of urine and incubating for four days. I tried to bring out the globular pearly white appearance of the colonies, and found it could best be done by using reflected light and a dark background. See also photographs of Surmin, February 1, further on.



Reflected light.



Direct light (same plate).

V. ON THE VITALITY OF *MICROCOCCUS MELITENSIS*
IN URINE (in which it has been excreted), ON CLOTH,
IN DUST, STERILE TAP WATER, AND STERILE MILK.

(Being Experiments 1 to 6, suggested by the Sub-Committee.)

By J. CRAWFORD KENNEDY, Capt. R.A.M.C., Member Mediterranean
Fever Commission. Malta, April, 1905.

Experiments 1 and 2.

How long does the *M. melitensis* retain its vitality in urine?

The following procedure was adopted:—A batch of urines was collected every day in sterile test-tubes plugged with cotton wool, and after $\frac{1}{4}$ c.c. from each had been planted out on plates they were laid aside. The next day, and every day till the 4th day, $\frac{1}{4}$ c.c. was again planted out on Petrie dishes. On the 4th day the plates made on the 1st day were sufficiently incubated, and the presence or absence of *M. melitensis* was noted. Those urines from which *M. melitensis* was absent were then discarded.

It was soon found that *M. melitensis* could be recovered after four days in urine, so the urines were left undisturbed for the four days until it was determined which samples contained *M. melitensis*. Those samples were then plated out day after day, the more plates being used each day according to the length of time the urine had been kept.

As the majority of the samples contained *M. melitensis* in very small quantities, the urine was disturbed as little as possible, so that, supposing the *M. melitensis* had been found in one sample on the 5th day, the next sample of urine was not plated out till the 6th day, and so on. In this way 525 samples of urine were gone through. In 53 of these *M. melitensis* was recovered in the first instance, but in only 12 was it recovered a second time.

The following table (p. 72) shows at a glance the result of the examination of these 12 samples.

The *M. melitensis* has therefore been recovered from urine 16 days old. The points that favour its existence in urine are—

1. Acidity,
2. Absence of other organisms, whether acid or alkaline.

1. The urine of Mediterranean Fever patients is markedly acid, and if moderately free from contaminating germs will remain so for a

| Date. | Name. | Number of colonies of <i>M. melitensis</i> found on 1st day per plate. | Result of examination of urine after standing the number of days indicated. + = <i>M. melitensis</i> recovered. | | | | | | | | | | | | | | | | | | | | | | |
|--|--------------|--|--|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 |
| Dec. 7 17 20 21 | Kinsella ... | 4 | + | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Bolt..... | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gane | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gane | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Bolt..... | 14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 27 31 Jan. 1 10 13 Feb. 1 13 | Gane | 70 { A B C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gane | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gane | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Walker ... | 29 (alkaline) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Gane | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Feb. 1 13 | Surmin ... | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Surmin ... | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

very long time. Both the urines in which *M. melitensis* was recovered on the 16th day were acid on that day. The urine of Gane remained acid 29 days.

Two other samples (Kinsella, December 7 and 11, 1904) were tested daily for acidity to litmus; the sample of December 7 began to turn alkaline on January 27, and the other on January 26—i.e., 51 and 46 days respectively. At the same time these samples, when planted out on plates, were found to be very filthy by the 5th or 6th day.

In only one sample was *M. melitensis* recovered, when the urine gave an alkaline reaction—viz., Walker sample, January 10. This urine was alkaline on the 1st day, and *M. melitensis* was recovered after it had stood for six days.

2. The presence of other organisms in the urine in any quantity is fatal to the recovery of *M. melitensis*. I am inclined to think that it may exist alongside the others, but that on nutrient media the excessive acidity or alkalinity produced by these rapid growing organisms prevents its development.

On several occasions I have found colonies of *M. melitensis* in a very acid plate hiding under the shelter (as it were) of a large alkali producing colony, where the acid is neutralised by the alkali.

Colonies that grow under these difficulties are always very tiny, do not have the amber colour by transmitted light, and in salt solution tend to remain in chains; the subcultures, however, are typical.

The sample of Surmin, February 1, was one of the two which contained *M. melitensis* in enormous quantities, and should have been an excellent sample for this experiment. *M. melitensis* was easily recovered from it on the 3rd day, but could not be isolated on the 6th, as the plate was overgrown with acid streptococci.

I attach a photograph (Plate 2) of the plates made from this sample on the 1st and on the 3rd day. It will be noticed that they are practically pure cultures of *M. melitensis*. The second plate is not so vigorous a growth as the first, the colonies tend to be smaller and less well defined, though their number is just as great as in the first. Both were incubated for four days. I have taken them in such a way that the light is reflected from the surface of the colonies, which thus stand out in relief.

Experiment with Artificially Infected Urine.

On December 16, 1904, the *M. melitensis* from the urine of a patient was added to the freshly passed urine of another, until the urine became cloudy. This was allowed to stand in a sterile test tube plugged with cotton-wool.

M. melitensis was easily recovered up to December 22 (six days). On the 23rd the growth of *M. melitensis* on the plate was beginning to get faint.

On the 24th the growth of *M. melitensis* was merely a faint blue haze along the edge of the track left by the drops of urine as they ran over the plate.

On the 26th *M. melitensis* could not be recovered as the plates were quite overgrown with acid streptococci. The urine remained acid for six days, after when the experiment was stopped.

Results.—The *M. melitensis* retains its vitality in urine (naturally infected) for 16 days, provided the urine remains acid and is fairly free from contaminating organisms.

It has been isolated from a urine (naturally infected) which had turned alkaline after six days.

In an artificially infected urine *M. melitensis* retained its vitality for seven days.

Experiments 3, 4, 5 and 6. Suggested by the Sub-Committee.

In carrying out these experiments, the great difficulty has been to know when one is dealing with a urine that contains the *M. melitensis*.

A urine collected one day and planted out on Petrie dishes has to be kept four days before the presence or absence of *M. melitensis* can be ascertained. By this time, even if *M. melitensis* be found in the plates, it is no guarantee that it has not died out by the 4th day, and in any case the vitality of any survivors has probably been seriously impaired.

To overcome this difficulty, I looked round for cases that would give a fairly regular supply of infected urine. In this I was fairly fortunate, and then my procedure was as follows:—

A sample of urine was collected every day from these patients, and every day it was—

1. Plated out on nutrose glucose litmus. This served as a control.
2. Allowed to dry on pieces of khaki drill.
3. Mixed with sterile dust.
4. Added to sterile tap water.
5. Added to sterile milk.

On the 3rd and 4th days the control plates were examined, and if *M. melitensis* was present the Experiments (2, 3, 4, 5) were proceeded with. This meant increasing the work greatly, as many samples proved useless.

The majority of samples treated in this way, and proved to be infected, contained *M. melitensis* in very small quantities—i.e., 4 to 30 colonies per cubic centimetre urine. It will be readily understood that the chances of recovering it again after diluting the sample 100 times are very small.



Urine, Surmin, February 1.

1. $\frac{1}{4}$ c.c. plated out on Petrie dish (nutrose-glucose-litmus-agar) on February 1, incubated four days.
2. $\frac{1}{4}$ c.c. plated out on February 4 and incubated four days.

I.

Experiment 4.

How long does *M. melitensis* in urine, when dried on cloth, retain its vitality?

A. A series of experiments was first made with urine artificially infected by *M. melitensis*. The method adopted was as follows:—

The cloth used was khaki drill, thoroughly sterilised and cut up into small pieces of $\frac{1}{2}$ inch square. One-quarter of a cubic centimetre of the infected urine was then placed on each piece of cloth, and allowed to dry naturally. At varying intervals a piece was teased out in sterilised water, and the water planted out on a series of Petrie dishes containing the nutrient medium. This was found to be the best way of recovering the *M. melitensis*, as, if the urine-contaminated cloth was first treated in broth, the rapid-growing organisms would render its recovery impossible.

(1) January 8. *M. melitensis* recovered from urine added to Mediterranean Fever urine 24 hours old with acid reaction. Procedure as above, except that the cloths were put in the incubator for six hours at 37° C. to dry the quicker.

Series of plates were made on January 10, 12, 14, and 16 from pieces of the cloth. *M. melitensis* was not recovered on any of these days; the plates were found to be very acid, and overgrown.

On the 17th another piece was teased out in sterile distilled water, and phenol phthalein added as an indicator. It required two drops of $n/10$ alkaline solution to render the mixture alkaline to the indicator. Two more drops of the alkaline solution were added, and then the mixture plated out on a series of Petrie dishes. The result was that one colony of *M. melitensis* was found in one of the plates. This colony was put through the tests, and proved to be *M. melitensis*. This experiment was repeated with another cloth on January 23, but without success.

Result:—*M. melitensis* found living in a very filthy cloth after nine days.

(2) January 16. An experiment similar to the preceding was started, with this difference that the urine was fresh and it was allowed to dry naturally. A control plate was made from the infected urine, and the cloth examined on January 17, 18, and 23. The result in every case was nil, the plates all being very foul and acid.

(3) January 20. Another urine was artificially infected and the same procedure carried out. A control was made from the urine on the 1st day and *M. melitensis* was easily recovered.

The cloth was examined on the 21st and 25th and *M. melitensis* was easily recovered on these days.

In contrast to the other two experiments the plates were very clean and comparatively free from contaminations.

The cloth of the 21st provided *M. melitensis* in great quantities, that of the 25th rather scantily.

On February 1 another cloth was examined. No *M. melitensis* was recovered, the plates were very clean.

On February 3 the cloth gave a few colonies of *M. melitensis*, this was the 14th day of the experiment. I continued examining these cloths every other day but never found *M. melitensis* again; the plates were always very clean, so that there was no chance of its being hidden by other organisms as was the case in the two former experiments.

Result.—*M. melitensis* found alive after 14 days.

B. The carrying out of this experiment with a naturally infected urine was very difficult and disheartening; it was only after many failures that I obtained a suitable urine.

This was urine of Surmin, February 1; it was not one of the samples which I had been using in the series of cloth experiments and so had not been put on to cloth on the 1st day. But having found *M. melitensis* in great quantities after the plate had been incubating three days and having fortunately kept the sample, I was able to put it on cloth when it was three days old; at the same time I made a control plate from this urine, and by looking to the report dealing with the vitality of *M. melitensis* in the urine, the photographs of this control plate and the plate of the 1st day will be seen side by side, showing the presence of *M. melitensis* in enormous quantities.

February 4. Urine of Surmin, February 1, put on cloth. Control as above.

February 7. Cloth examined. *M. melitensis* recovered in great quantities.

February 17. Cloth examined. No *M. melitensis* recovered.

February 21. Cloth examined. One colony of *M. melitensis* recovered. This colony was of a very dark amber colour. It answered all the tests for *M. melitensis* perfectly. I continued for some days making plates from the cloth but got no further recovery, though everything was favourable, as the plates showed very little growth.

Result.—*M. melitensis* excreted in urine dried on cloth will retain its vitality for 17 days, though it tends to die out before the 13th day.

It should be remembered that this sample of urine had stood for three days before being put on cloth, so that the vitality of the *M. melitensis* had probably been impaired.

II.

Experiment 3.

How long does *M. melitensis* retain its vitality in dust moistened with infected urine?

Procedure.—Dust used was the dust and mud from the road, which,

when dry, blows about as a very fine powder. It was thoroughly sterilised in hot air chamber. The infected urine was mixed up with it until it became of a pasty consistency. This was allowed to dry at room temperature and when dry was examined for *M. melitensis*. A small quantity of the dust was mixed up with sterile distilled water, thoroughly shaken, and the fluid pipetted off and planted out on plates. The dust generally was so fine that it would form an emulsion in the water and the whole mixture could be planted out. In this way it was found that *M. melitensis* could very readily be recovered.

A. A series of experiments was first performed with dust and urine which had been artificially infected with *M. melitensis* recovered from urine. The first two of these were unsuccessful owing to the very filthy state of the urine used. The third gave the following result:—

January 20. *M. melitensis* recovered from urine added to Mediterranean Fever urine; resulting emulsion added to dust which was allowed to dry at 16° C.

January 21. Small quantity plated out. *M. melitensis* in great quantities recovered on 3rd day, still more on the 4th.

January 25. Small quantity planted on one plate; same result as on 21st. *M. melitensis* recovered.

February 3. Another plate made. *M. melitensis* recovered in good quantities.

February 9 (20th day). Three plates made. Two contained *M. melitensis* in good quantity; one contained none. All the plates were very clean.

February 17 (28th day). Two plates made. *M. melitensis* recovered from both in great quantity.

February 25 (36 days). Two plates made. No *M. melitensis* was recovered from these plates.

March 1 (41 days). Six plates were made. *M. melitensis* was recovered from only two plates and in very small numbers.

March 4 (44 days). Seven plates made. Two of these each contained one colony of *M. melitensis*. This finished the supply of infected dust.

Result.—It is evident from this that the *M. melitensis* retained its vitality in the dust with no difficulty for one month, but after that time it died out quickly, but could be found when large quantities of dust were used and many plates made up to the 44th day.

B. Series of experiments were then made with urines naturally infected on the lines laid down in the beginning of this Report, viz., mixing consecutive series of fresh samples of urine with dust and then waiting until the controls were positive or negative for the presence of *M. melitensis*.

(1) In the first series *M. melitensis* was found in five controls, but only in quantities of 1 to 8 colonies per $\frac{1}{4}$ c.c. These dusts were plated out frequently and in large quantities, but no recovery of *M. melitensis* was made.

(2) In the second series 12 controls contained *M. melitensis*, but no *M. melitensis* was recovered from the dust. The number of colonies of *M. melitensis* in the controls of this series varied from 1 to 13 per $\frac{1}{4}$ c.c.

(3) On February 4 it was found that a sample of Surmin's urine passed on February 1 contained *M. melitensis* in large quantities (see Report on Vitality in Urine and Cloth Experiments), and some of it was mixed with dust and allowed to dry at room temperature.

February 5. Dust was dry and some was planted out on three plates. *M. melitensis* was recovered from each of these plates in great quantities, first appearing on the 3rd day.

Judging from the experiment with dust and artificially prepared urine, I allowed this dust to stand undisturbed till February 17 (13th day of experiment). On this day I again planted out some of the dust, but could not recover *M. melitensis*. I again planted it out on February 21, 25, and March 1, but could not recover it again.

Result.—Dust contaminated with naturally infected urine contained great quantities of *M. melitensis* after 24 hours, but after 13 days the *M. melitensis* had completely died out.

Note.—The same remark applies here as in the cloth experiment, viz., that the urine was three days old before being put in dust. Consequently, the vitality of *M. melitensis* may have been considerably impaired.

Experiments with Unsterilised Soil.

A red soil obtained from a garden bed was used. The method adopted was the same as for sterilised dust, but the proportion of sterile water to soil was greater. The urines used were the same as used in the second series of dust experiments. Plates were obtained in which the colonies were fairly discrete, but no *M. melitensis* was recovered.

III.

Experiment 5.

To determine vitality of *M. melitensis* in infected urine when added to sterile tap water. The method of procedure was as indicated above.

Fresh samples of urine were added to the sterile water contained in flasks of 100 c.c., or in test-tubes of 10 c.c.

One c.c. of urine was added to 100 c.c. and $\frac{1}{4}$ c.c. to 10 c.c. Controls were at the same time taken from each sample of urine and if *M. melitensis* was found the experiment was proceeded with.

In this way 20 samples of urine were tried, five in flasks and 15 in test-tubes.

Of those put in flasks three were found to contain *M. melitensis* in small quantities (viz., 72, 8, and 4 colonies per cubic centimetre). These three flasks were plated out on 4th, 7th, 8th, 14th, 15th, 17th, and 25th days. *M. melitensis* was not recovered.

Of those put in test-tubes, two were found to contain *M. melitensis* (4 and 12 per cubic centimetre). These were plated out on the 5th, 8th, 12th, and 15th days. *M. melitensis* was not recovered.

Being so unsuccessful with the experiment conducted with naturally infected urine, I then carried it out with urine artificially infected with *M. melitensis*.

February 14. The urine used was that of a Mediterranean Fever patient, freshly drawn, and the *M. melitensis* used had also been isolated from the urine of a Mediterranean Fever case.

The *M. melitensis* was added to the urine until a milkiness was visible. One c.c. of this emulsion was added to a flask containing 100 c.c. of sterile tap water and the flasks allowed to stand in the laboratory cupboard, where there was practically a constant temperature of 15° C.

On the following days, $\frac{1}{4}$ c.c. of the sediment was plated out on Petries containing nutrose-glucose-litmus-agar, February 15, 16, 17, 18, 19, 21, 23 and every day to March 4, 6, 11, 15. *M. melitensis* was recovered up to and on March 6, being the 20th day of the experiment.

For the first four days only one plate was taken from the flask, and the result was as follows:—On February 15 (the first completed day) the *M. melitensis* was recovered in great quantity, the first appearance being noticed on the 17th (48 hours' growth); on February 16 only five colonies appeared; on February 17 one only, and on the 18th none. Thereupon three plates were made each day and *M. melitensis* was always recovered from one or two of them.

The plates taken from February 21 to March 11 all contained numbers of colonies which were practically identical with *M. melitensis* to the naked eye by transmitted light, but by reflected light were a dull white and opaque instead of having a greenish blue halo appearance. This organism was a coccus slightly larger than *M. melitensis*, and which tended to remain in chains of four and five when emulsified in salt solution.

This organism gave a great deal of bother, especially towards the end of the experiment, as I found it increasingly difficult to separate the *M. melitensis* from it. When fishing a *M. melitensis* colony from the plate, and feeling certain that nothing else had been touched, I found that the sub-culture was more frequently than not contaminated by this organism, which in the sub-culture on nutrose-glucose-litmus grew with the production of acid. It was generally necessary to sub-culture twice before obtaining a pure growth of *M. melitensis*.

This organism when sub-cultured was an acid producing streptococcus not agglutinated by Mediterranean Fever serum and partly losing its stain when treated by Gram's method.

On March 11 and 15 no *M. melitensis* was recovered and the plates made on the latter date were very dirty, being overgrown with rapid growing alkaline colonies.

Conclusions. That *M. melitensis* tends to die out quickly in sterile tap water, but can be recovered from it up to the 20th day.

Experiment 6.

To determine survival of *M. melitensis* in infected urine which has been added to sterile milk. This experiment was carried out on the same lines as the former. In every case test-tubes containing 10 c.c. of sterile milk, to which a little litmus had been added, were used; the amount of urine added to each test-tube of milk was $\frac{1}{4}$ c.c.

December 12. Six samples of urine added to six litmus milk tubes. Control: no *M. melitensis* found in any one of the samples.

December 13. Five samples urine added to milk. Control: one sample (Kinsella) contained one colony *M. melitensis* per cubic centimetre. Four $\frac{1}{4}$ c.c. of the mixture of milk and this infected sample were plated out on four nutrose-glucose-litmus-agar plates on December 18, 24, 27. No *M. melitensis* was recovered.

December 14. Six samples urine added to milk. Control: one sample (Kinsella) contained two colonies *M. melitensis* per cubic centimetre. The infected milk was plated out on December 21, 24, and 27. No *M. melitensis* recovered.

December 19. Three samples urine added to milk. Control: none contained *M. melitensis*.

December 20. Ditto. Five samples of urine.

December 21. One sample urine from Boy Bolt. Control found to contain 56 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days: December 27 (6th day of experiment). Milk showed no coagulation and remained alkaline. *M. melitensis* recovered.

December 31 (10th day of experiment). Milk still alkaline, no coagulation. *M. melitensis* recovered.

January 4 (14th day) }
January 8 (18th day) } Milk alkaline, *M. melitensis* not recovered.

January 12 (22nd day). Milk turned acid, *M. melitensis* not recovered.

December 24. Five samples of urine added to milk. Control: Bolt found to contain 16, and Gane 20 colonies *M. melitensis* per cubic centimetre. Bolt had turned the milk acid on the 31st. *M. melitensis* not recovered. Gane remained alkaline till January 4. *M. melitensis* not recovered.

December 24. Four samples urine added to milk. Control: one sample (Gane) was found to contain 280 colonies *M. melitensis* per cubic centimetre. The milk was plated out on the following days:

| | |
|-----------------------|--|
| December 31 (4th day) | } Milk remained alkaline all this time. <i>M. melitensis</i> was recovered on each of these days. |
| January 4 (8th ") | |
| " 8 (12th ") | |
| " 12 (16th ") | } By the 20th day (January 16) milk had turned acid. No <i>M. melitensis</i> was recovered after this. |
| January 16 (20th day) | |
| " 20 (24th ") | |
| " 24 (28th ") | |

After having recovered the *M. melitensis* up to the 16th day, it was decided to carry on the experiment without touching the infected milk till the 17th day of the experiment.

January 21. One sample of urine added to milk. Control gave no *M. melitensis*.

January 22. One sample of urine, ditto.

January 24. One sample urine added to milk. Control contained eight colonies *M. melitensis* per cubic centimetre. Milk plated on following days:—

| | |
|--------------------------------------|---|
| February 10 (17th day of experiment) | } Milk remained alkaline. <i>M. melitensis</i> not recovered. Milk turned acid. <i>M. melitensis</i> not recovered. |
| " 14 (21st " ") | |
| " 18 (25th " ") | |

January 25. One sample of urine added to milk. Control contains four colonies per cubic centimetre. Plated out February 12 and 18. *M. melitensis* not recovered.

January 27. Same as January 25. Plated out on February 14, *M. melitensis* not recovered.

January 29. One sample urine. Control contained no *M. melitensis*.

January 30. Same as January 25. Plated out February 16 and 22. No *M. melitensis* recovered.

Six more samples were added to milk in the same way on February 4, 5, 6, and 7, but the controls were negative.

The *M. melitensis* recovered above was proved by the usual tests.

The only thing to be noted as regards cultural or other appearance is that in plates of the 12th and 16th days the colonies were of a darker amber colour than usual.

Summary.

Forty-eight samples of urine were added to milk; control experiments proved the presence of *M. melitensis* in 10 of them. *M. melitensis* was recovered from two of these; in one up to the 10th day and in the other up to the 16th day. In both cases the milk remained alkaline as long as the *M. melitensis* was recovered.

These urines must have been very clean and comparatively free from acid organisms. In most cases the milk became curdled in four days' time.

Conclusions.—*M. melitensis* will live in sterile milk which has been contaminated by infected urine as long as the milk remains alkaline or neutral to litmus.

Summary.

M. melitensis in the condition in which it is excreted in urine will retain its vitality—

1. Dried on cloth for 17 days.
2. In dust: for less than 13 days.
3. In sterile tap water. Not recovered.
4. In sterile milk for 16 days.

M. melitensis derived from urine, grown on media and then added to Mediterranean Fever urine, retains its vitality—

1. Dried on cloth for 14 days.
 2. Dried in dust for 44 days.
 3. Mixed with sterile tap water for 20 days.
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VI. A PRELIMINARY NOTE ON THE EXAMINATION OF THE BLOOD OF GOATS SUFFERING FROM MEDITERRANEAN FEVER.

By Dr. T. ZAMMIT, Member of the Mediterranean Fever Commission.

On June 14, as detailed by Major Horrocks, I examined the blood of six goats, which were brought to the lazaretto on June 12, and obtained the following results :—

Goat No. 1.—Strong immediate reaction, in dilution of 1 to 20.

Goat No. 2.—Strong immediate reaction, in dilution of 1 to 20.

Goat No. 3.—Strong reaction, after half-an-hour.

Goat No. 4.—No reaction.

Goat No. 5.—Strong reaction, after half-an-hour.

Goat No. 6.—Strong immediate reaction.

On June 15 the bloods were again examined, with identical results.

On June 18 about 5 c.c. of blood were taken from Goat No. 6, and distributed in six broth-tubes. On June 25 passages from the broth-tubes were made on to agar slopes, and the *M. melitensis* recovered in pure culture. This micro-organism was also recovered from the blood of Goat No. 5.

Blood has also been taken from Goats Nos. 1, 2, and 3, and distributed in broth-tubes as usual, but, so far, the *M. melitensis* has not been recovered.

Material from Abattoir.—Dr. Caruana Scicluna having suggested that possibly infected goats might be met with in the abattoir, I have examined 46 spleens removed with aseptic precautions, but, so far, have only recovered the *M. melitensis* from one. The blood from the goats was examined for agglutination with the *M. melitensis*, and a definite reaction was obtained in seven.

(For further details, see 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

VII. PRELIMINARY NOTE ON GOATS AS A MEANS OF PROPAGATION OF MEDITERRANEAN FEVER.

By Major W. H. HORROCKS, R.A.M.C., Member of the Mediterranean Fever Commission.

(Reprinted from the 'Proceedings of the Royal Society,' Series B, vol. 76, 1905, No. B 510.)

With the object of ascertaining, by experimental inoculation, whether goats could be infected by the *M. melitensis*, six goats were bought on June 12, 1905, from two different herds, and placed in the lazaretto. On June 14 Dr. Zammit, as a preliminary step to our experimental work, took blood from each of these goats, and proceeded to test the action of the serum on the *M. melitensis*. He found, to his great surprise, that the serum of five of the goats, when considerably diluted, caused agglutination of this microbe. On June 15 similar results being again obtained, Dr. Zammit brought specimens of the bloods to the Public Health Laboratory, and asked me to confirm his observations. I obtained the following results:—

- Goat No. 1.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*, visible to the naked eye. When diluted 1 to 100, however, the serum gave no reaction.
- Goat No. 2.—Blood serum diluted 1 to 10 and 1 to 40 caused immediate agglutination of the *M. melitensis*. A dilution of 1 to 100 produced a complete reaction after 15 minutes.
- Goat No. 3.—Blood serum diluted 1 to 10, 1 to 40, and 1 to 100, caused immediate agglutination of the *M. melitensis*, but, in the case of the dilution 1 to 100, the clumps were not visible to the naked eye until after 15 minutes.
- Goat No. 4.—The blood serum produced no reaction with the *M. melitensis*.
- Goat No. 5.—The blood serum diluted 1 to 10 caused immediate agglutination, but dilutions of 1 to 40 and 1 to 100 did not produce a complete reaction until after 15 minutes.
- Goat No. 6.—Blood serum diluted 1 to 300 caused complete agglutination of the *M. melitensis*, visible at once with the naked eye.

The reactions thus obtained, and especially that of Goat No. 6, suggested that possibly five of the goats were suffering from Mediterranean Fever, acquired under natural conditions. The goats were stated to be healthy, but were sold cheaply, as they had given very little milk for some time. They were bought from pens in the neighbourhood of Birchircara and St. Julians, and taken straight to the lazaretto, where they were placed in clean stalls, which had never been used for any experimental work with the *M. melitensis*.

Dr. Zammit and I then arranged to make a complete study of these animals; Dr. Zammit undertook the investigation of the blood, and I made myself responsible for the bacteriological examination of the milk and urine.

Bacteriological Examination of Milk and Urine obtained from Naturally Infected Goats.

Goat No. 6.—I commenced work with this goat, as its blood serum, when diluted 1 to 300, caused immediate agglutination of the *M. melitensis*. The animal did not appear well, and had a very poor coat. The udders were flaccid, but the milk exuded appeared normal in character. The temperature was taken morning and evening, and compared with that of a healthy goat. The evening temperature never rose above 103°, and, as this temperature is often recorded in the case of perfectly normal goats, a febrile temperature could not be said to be present. On June 18 milk was withdrawn, and 1 c.c. centrifugalised; the deposit was then carefully spread over 10 litmus-nutrose-agar plates. After four days' incubation at 37° C., colonies of the *M. melitensis* appeared in every plate. The colonies were at once tested with a dilute (1 to 100) specific serum obtained from an inoculated rabbit. The micrococci were found to agglutinate at once, the clumps being visible to the naked eye. Some of the colonies were then planted out on agar slopes, and the resulting growths, when subjected to the usual confirmatory tests, showed that the *M. melitensis* was undoubtedly being excreted in the milk of this goat.

On June 22 the milk was again examined and the *M. melitensis* recovered once more.

On June 23 examination of the urine was commenced. The vagina was washed out with an antiseptic solution and a catheter, previously sterilised in boiling water, passed into the bladder. The urine so obtained was plated on litmus-nutrose-agar, but after four days' incubation at 37° C., in spite of the precautions taken, the plates were found densely crowded with saprophytic organisms, and the *M. melitensis* could not be detected.

On June 24 and 26 the urine was again plated, the same precautions being used, but the plates were densely crowded with foreign organisms and the *M. melitensis* could not be seen.

On June 27, 28, 29, and 30, and on July 1, 3, 4, 5, 7, 8, 9, and 10, the urine was also examined, but up to the present the *M. melitensis* has not been recovered.

The milk was plated again in June and July, and the *M. melitensis* was found on each occasion.

Result.—The *M. melitensis* appears to be steadily excreted in the apparently normal milk of this goat, but up to the present it has not been found in the urine.

Goat No. 1.—This animal appeared healthy, but the udders were flaccid, and the milk exuded had a thin serous appearance. The temperature was taken regularly, but no indications of fever were observed.

On June 22, 1 c.c. of the milk was centrifugalised and the deposit plated. After four days' incubation at 37° C., the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were obtained.

On June 26, 29, and 30 the urine was examined, but no signs of the *M. melitensis* could be discovered.

On July 1, 10 c.c. of the urine were centrifugalised and the deposit plated; four days later every plate was found studded with colonies of the specific microbe. The colonies were fished, planted on agar slopes, and the resulting growths tested in the usual manner.

Result.—The *M. melitensis* is excreted in very large numbers in the serous-looking milk of this goat. It is also excreted in the urine.

Goat No. 2.—This goat appeared quite well, and the milk exuded from the udders had a normal appearance. There were no indications of fever.

On June 22, 1 c.c. was centrifugalised and the deposit plated. After four days' incubation about 30 colonies appeared in every plate. On June 24 and 26 the milk was again examined, and colonies of the *M. melitensis* were recovered on both occasions.

The urine was examined on June 23, 26, 27, 28, 29, and 30, and on July 1, 3, and 6, but the *M. melitensis* could not be detected.

Result.—The *M. melitensis* appears to be excreted in small quantity in the normal-looking milk of this goat. It has not yet been detected in the urine.

Goat No. 3.—This goat looked healthy and had no fever, but its milk was thin and serous. On June 22 the milk was examined, one loopful of the serous milk being spread over each plate. After four days' incubation all the plates were found so densely crowded with colonies of the *M. melitensis* that an accurate count could not be made.

On June 24 and 26 the milk was again examined and similar results were again obtained.

The urine was examined on June 23, 26, 28, and 30, and on July 1, 3, 6, 8, 9, 10, 11, but no signs of the *M. melitensis* could be discovered.

Result.—The *M. melitensis* appears to be present in enormous quantities in the thin serous-looking milk of this goat, but it has not yet been found in the urine.

Goat No. 5.—This goat was in poor condition, the udders were flaccid, and the milk exuded had a thick jelly-like appearance.

On June 22 the milk was examined, one loopful of the jelly-like material being spread over each plate. After four days incubation all the plates were covered with minute colonies of the *M. melitensis*. On June 24 and 26 the milk was again examined, and densely crowded plates were obtained as before.

On June 25 and 30 the urine was examined, but no colonies of the *M. melitensis* were detected.

On July 1 the urine was again plated, and four days later every plate was found to contain numerous transparent colonies strongly resembling those of the *M. melitensis*. Some of the colonies were fished and planted out on agar slopes. The resulting growths were then subjected to the usual confirmatory tests, and the *M. melitensis* proved to be undoubtedly present.

The five goats just examined being considered by their owners to be "out of milk," would not be likely to be employed for milking purposes, though in the case of Goats Nos. 2 and 6, the milk might easily have been used without any fear of suspicion arising as to its being abnormal. Consequently it appeared very desirable to examine the herds which were actually supplying milk to Valetta, Sliema, and the various hospitals.

I therefore asked Captain Kennedy, R.A.M.C., to visit the various herds, and, with the owners' consent, take blood from the ears, and test the action of the sera on the *M. melitensis*. The results he obtained are given in Part VIII; it will be seen that, out of 161 goats examined, 84 gave a reaction, corresponding to a percentage of 52 probably infected with Mediterranean Fever. I then obtained samples of milk from some of the apparently infected animals, and proceeded to plate them on litmus-nutrose-agar. The following results have been obtained up to the present time :—

Examination of the Goats supplying Milk to Forrest Hospital.

I visited this herd, which assembles outside the hospital gate every morning, and selected Goats Nos. 38, 48, 37, and 43 from Captain Kennedy's list.

Goat No. 38.—The milk from this animal was centrifugalised, and the deposit plated on July 4, 5, 6, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Goat No. 48.—The milk was examined on the same dates as Goat No. 38, but, so far, the *M. melitensis* has not been isolated.

Goat No. 37.—The milk of this animal was taken on July 4, and 2 c.c. centrifuged; the deposit was then plated. After four days' incubation every plate was found densely crowded with small colonies of the *M. melitensis*; the colonies were so numerous that it was impossible to make an accurate count. The colonies were fished and planted on agar, the growths resulting responded to all the tests characteristic of the *M. melitensis*.

On July 5 and 6 the milk was again plated, and similar results were obtained.

As this goat was in full milk, there cannot be any doubt that the *M. melitensis* was being excreted in large numbers. A pint of the milk was then collected, and Dr. Zammit very kindly made a chemical examination of the sample. The result given below shows that the milk was of good quality.

Analysis of Milk from Goat No. 37.

| | |
|------------------------|---------------|
| Density at 15° C. | 1030 |
| Fat | 4·3 per cent. |
| Total solids | 13·18 „ |
| Solids, non-fat | 8·8 „ |
| Ash | 0·51 „ |

Goat No. 43.—The milk of this goat was examined on July 4, 5, 6, 7, 8, 9, and 10, but, up to the present, the *M. melitensis* has not been isolated.

A reference to Captain Kennedy's list shows that, while Goat No. 37 reacted in a dilution of 1 to 60, Goats Nos. 38, 48, and 43 only reacted in a dilution of 1 to 20, and were probably in an early stage of the disease.

Examination of a Small Herd supplying Milk to Valetta Station Hospital.

Goats Nos. 27, 30, and 32 were selected from this herd. The goats were kept at Casal Curmi, and brought every morning to the Station Hospital.

Goat No. 30.—On June 29 and 30 milk was centrifuged and plated in the usual manner, but the *M. melitensis* was not detected.

On July 1 plates were again made, and a few typical colonies appeared.

On July 3 10 c.c. of the milk were centrifuged, and the deposit plated; four days later every plate was found densely crowded with colonies of the *M. melitensis*.

On July 6 similar results were obtained.

A sample of the milk was then analysed by Dr. Zammit, and found to have an average chemical composition.

Goats Nos. 27 and 32.—The milk from these goats was examined on June 29 and 30, and on July 1, 3, 7, 8, and 10, but, up to the present, the *M. melitensis* has not been isolated.

Examination of a Small Herd Supplying Milk to Valetta.

This herd assembled in St. John's ditch, and 17 out of 25 animals showed a blood reaction with the *M. melitensis*, and six of them reacted when the serum was diluted 1 to 100. Goats Nos. 50 and 52 were selected from Captain Kennedy's list.

Goat No. 50.—On July 6, 1 c.c. of the milk was centrifugalised and the deposit spread over the usual plates. Four days later all the plates were found densely crowded with small colonies of *M. melitensis*.

The confirmatory tests were applied in the usual manner. This animal was considered one of the best milkers in the herd, and its owner valued it at £5, whereas the ordinary price for a goat in milk varies from £3 to £4.

Goat No. 52.—This animal appeared in good health and its udders were full of milk. It was purchased and placed in the lazaretto.

On July 5 milk was withdrawn and 1 c.c. centrifugalised; the deposit was then spread over nutrose-agar plates in the usual manner. After four days' incubation at 37° C., all the plates were found so crowded with colonies of *M. melitensis* that a reliable count could not be made.

On July 6 and 8 the milk was again examined and similar results were obtained.

A sample of the milk was submitted to Dr. Zammit for chemical analysis; he obtained the following results:—

Specific gravity at 15° C..... 1031

Total solids, 14.0 per cent. ; fat, 3.6 per cent. ; ash, 0.73 per cent.

Examination of a Herd Supplying Milk to Sliema.

Two goats were bought from this herd and placed in the lazaretto. The pens were in the neighbourhood of Misida.

Goat No. 15.—On July 5 the blood was examined and the serum, diluted 1 to 50, was found to cause complete agglutination of the *M. melitensis* visible to the naked eye. The goat appeared to be in good health, and the udders were full of milk. Some milk was withdrawn and 2 c.c. centrifugalised; the deposit was then plated in the usual manner. On July 9 the plates were found covered with small colonies of the *M. melitensis*.

On July 6 the milk was again examined, and the deposit from

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1 c.c. produced as before an immense number of colonies of *M. melitensis*.

The urine was withdrawn by a catheter and plated on July 5, 6, 7, 8, 9, and 10, but up to the present the *M. melitensis* has not been isolated.

A chemical analysis of the milk was made by Dr. Micallef, with the following results :—

Total solids, 13·5 per cent. ; fat, 4·1 per cent. ; ash, 0·75 per cent.

Goat No. 16.—This goat was taken from the same herd as No. 15. On July 4 the blood was examined, and the serum diluted 1 to 60, was found to cause immediate clumping of the *M. melitensis*. The milk and urine have been examined daily since July 4, but up to the present the *M. melitensis* has not been isolated from either source.

Conclusions.—The results obtained show that some of the goats in every herd examined are suffering from Mediterranean Fever. The *M. melitensis* is exuded in the milk in enormous numbers when the disease has been present sufficiently long to cause a change in the physical characters of the fluid. It is also excreted in considerable numbers even when the animals are in “full milk,” and no changes have occurred in either the physical or chemical characters of the milk.

The *M. melitensis* is also excreted in the urine of goats suffering from Mediterranean Fever, but up to the present it has only been found when the disease has existed for some time and physical changes have occurred in the milk.

VIII. EXAMINATION OF GOATS' BLOOD FOR REACTION TO MEDITERRANEAN FEVER.

By J. CRAWFORD KENNEDY, R.A.M.C., Member of the Mediterranean Fever Commission, Malta.

| No. of herd. | Owner and number in each herd. | Address. | Milk supplied to— | Total number examined. | Number that gave no reaction. | Number that reacted to Med. Fever. | Per. centage of reactions. | Table showing amount of reaction in each infected goat by dilutions up to 100. | | | | | | |
|--------------|--------------------------------|--------------------------------------|---|------------------------|-------------------------------|------------------------------------|----------------------------|--|-----|-----|-----|-----|-----|------|
| | | | | | | | | Dilution. | 10. | 20. | 40. | 60. | 80. | 100. |
| 1 | C—, Nos 1 to 4 and 74 to 83 | Casal Tar-shiel near C a s a l Paulo | Cottonera Hospital, Zabbar Gate and near lying part of town | 14 | 7 | 7 | 50 | No. of goat { | 2 | 74 | 1 | ... | ... | 83 |
| | | | | | | | | Total ... | 80 | 78 | 75 | ... | ... | ... |
| 2 | A—M— and F—G—, Nos. 5 to 17 | Zabbar ... | Cottonera Hospital, Zabbar Gate and near lying town | 13 | 12 | 1 | 7.6 | No. of goat { | ... | ... | ... | ... | ... | 5 |
| | | | | | | | | Total ... | ... | ... | ... | ... | ... | 1 |
| 3 | J—, Nos. 18 to 20 | Hamrun ... | Valetta ... | 3 | 3 | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 4 | J—F—, Nos. 21 to 23 | C a s a l Curmi | Valetta Hospital and town | 13 | 9 | 4 | 30.7 | No. of goat { | ... | 32 | ... | 32 | ... | 27 |
| | | | | | | | | Total ... | ... | 30 | ... | ... | ... | ... |
| | | | | | | | | Total ... | ... | 2 | ... | 1 | ... | 1 |

| No. of herd | Owner and number in each herd. | Address. | Milk supplied to— | Total number examined. | Number that gave no reaction. | Number that reacted to Med. Fever. | Per-centage of reactions. | Table showing amount of reaction in each infected goat by dilutions up to 100. | | | | | | | | |
|-------------|--------------------------------|------------------|----------------------|------------------------|-------------------------------|------------------------------------|---------------------------|--|-----------|------------------|-----|-----|-----|------|-----|----|
| | | | | | | | | Dilution. | 10. | 20. | 40. | 60. | 80. | 100. | | |
| | | | | | | | | | | | | | | | | |
| 5 | C—, Nos. 34 to 48 | St. George's | Forrest Hospital ... | 15 | 10 | 5 | 33·3 | { No. of goat | ... | 38 | ... | 37 | ... | ... | | |
| | | | | | | | | | Total ... | ... | 4 | ... | 1 | ... | ... | |
| | | | | | | | | | | { No. of goat | 51 | 54 | ... | 64 | ... | 50 |
| | | | | | | | | | | | 59 | 66 | ... | ... | ... | 52 |
| 6 | M— M—, Nos. 49 to 73 | St. John's Ditch | Valetta ... | 25 | 8 | 17 | 68 | { No. of goat | 62 | 67 | ... | ... | ... | 55 | | |
| | | | | | | | | | 63 | 70 | ... | ... | ... | 60 | | |
| | | | | | | | | | 65 | ... | ... | ... | ... | 68 | | |
| | | | | | | | | | 73 | ... | ... | ... | ... | 71 | | |
| 7 | G— M—, Nos. 84 to 129 | Hamrun ... | Valetta ... | 46 | 20 | 26 | 56 | { No. of goat | ... | 4 | ... | 1 | ... | 6 | | |
| | | | | | | | | | Total ... | 97 | 88 | 85 | 103 | ... | 99 | |
| | | | | | | | | | | 109 | 93 | 90 | 111 | ... | 102 | |
| | | | | | | | | | | 117 | 96 | ... | 119 | ... | 105 | |
| 120 | 106 | ... | 126 | ... | 121 | | | | | | | | | | | |
| 123 | 110 | ... | ... | ... | 122 | | | | | | | | | | | |
| ... | 112 | ... | ... | ... | 129 | | | | | | | | | | | |
| ... | 113 | ... | ... | ... | ... | ... | | | | | | | | | | |
| ... | 128 | ... | ... | ... | ... | ... | | | | | | | | | | |
| ... | 128 | ... | ... | ... | ... | ... | | | | | | | | | | |
| Total ... | 5 | 9 | 2 | 4 | ... | 6 | | | | | | | | | | |

| | | | | | | | | | | | | | | | | | | |
|---|-----------------------------|-------|-----|---------|-----|-----|----|---|----|----|----------------|-----------|-----|-----|-----|-----|-----|-----|
| 8 | F—V—, Nos. 130 to 161 | Pietà | ... | Valette | ... | ... | 32 | 8 | 24 | 75 | No. of goat | Total ... | 137 | 134 | 131 | 147 | ... | 13 |
| | | | | | | | | | | | | | 140 | 135 | 157 | ... | ... | ... |
| | | | | | | | | | | | | | 141 | 141 | ... | ... | ... | ... |
| | | | | | | | | | | | | | 148 | 149 | ... | ... | ... | ... |
| | | | | | | | | | | | | | ... | ... | ... | ... | ... | ... |
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| | | | | | | | | | | | | | ... | ... | ... | ... | ... | ... |
| | | | | | | | | | | | | | 4 | 4 | 2 | 1 | ... | 13 |

Examination of a Herd of Goats kept Privately and not allowed outside their own Field, as a Comparison with the Herds that Walk into Town Daily.

| | |
|------------------------------------|----|
| Total examined | 10 |
| No reaction | 5 |
| React to Mediterranean Fever | 5 |

Of the five which reacted :—

| | |
|-----------------------------|-----------------|
| 1 reacted in dilution | $\frac{1}{10}$ |
| 3 " " | $\frac{3}{20}$ |
| 1 " " | $\frac{1}{100}$ |

∴ The percentage of infected goats in this herd is 50 per cent., comparing very closely with 52 per cent. of the public herds.

IX. RESULTS OF EXAMINATIONS FOR THE ISOLATION OF *MICROCOCCUS MELITENSIS* FROM THE BLOOD, URINE, AND SPUTUM OF CASES INFECTED WITH MEDITERRANEAN FEVER IN HASLAR HOSPITAL.

By P. W. BASSETT-SMITH, Fleet-Surgeon, Haslar.

Blood.—The blood was obtained from the median basilic vein of the arm, which had been carefully sterilised; from 1 to 3 c.c. were taken with an all-glass anti-toxin syringe; the blood was at once injected into flasks containing 50 c.c. of peptone broth; from this sub-cultures were made on to agar daily for 14 days at least, the resulting growth being tested by—

- (1) Agglutination with specific serum.
- (2) Alkaline reaction with litmus milk.
- (3) Negative staining by Gram.

In all 27 bloods were examined from 24 patients, with 16 positive and 11 negative results, as shown in the following table:—

| No. | Day of disease. | Condition. | Temperature. ° F. | Amount of blood taken. c.c. | Result. |
|-----------------------------|-----------------|----------------------|----------------------|--------------------------------|----------|
| 1 | 50 | Acute relapse | 102·4 | 3 | Positive |
| 2 | 142 | Slight „ | 99·4 | 2·5 | „ |
| 3 | 84 | Acute „ | 102 | 3 | „ |
| 4 | 117 | „ „ | 103·4 | 3 | „ |
| 5 | 92 | „ „ | 102 | 3 | „ |
| 6 | 23 | Prim. wave | 104 | 3 | „ |
| 7 | 34 | Sec. „ | 103 | 3 | „ |
| 8 | 167 | Relapse..... | 102 | 1 | Negative |
| short relapse with neuritis | | | | | |
| 9 | 143 | Acute relapse | 101 | 2 | Positive |
| 10 | 44 | „ „ | 101·2 | 2·5 | „ |
| 11 | 105 | „ „ | 102·6 | 3 | „ |
| 12 | 153 | „ „ | 105 | 2 | „ |
| 13 | 44 | „ „ | 102·8 | 0·5 | „ |
| 14 | 41 | „ „ | 102 | 2 | „ |
| 15 | 80 | „ „ | 102·6 | 2 | „ |
| 16 | 111 | „ „ | 101 | 2 | „ |
| 17 | 61 | „ „ | 103 | 2 | „ |
| 18 | 122 | Convalescent | N. | 1 | Negative |
| 19 | 185 | Cachexia..... | N. | 2 | „ |
| 2 | 110 | Slight relapse | 100 | 3 | „ |
| slight wave, not repeated | | | | | |

| No. | Day of disease. | Condition. | Temperature. ° F. | Amount of blood taken. c.c. | Result. |
|-----|-----------------|--------------------|----------------------|------------------------------------|----------|
| 21 | 165 | Convalescent | N. | 3 | Negative |
| | | | | went out next day | |
| 22 | 159 | „ | N. | 3 | Negative |
| | | | | no return of the fever | |
| 23 | 130 | „ | N. | 3 | Negative |
| 24 | 78 | „ | N. | 3 | „ |
| | | | | no return of the fever | |
| 25 | 134 | „ | N. | 3 | Negative |
| | | | | no fever, great anæmia | |
| 26 | 120 | „ | 99 | 3 | Negative |
| | | | | flask contaminated on the 14th day | |
| 27 | 1 year | Cachexia | N. | 3 | Negative |

Two flasks after being inoculated were found to be contaminated and are not included in this list, the others were either sterile or contained a pure culture of *M. melitensis*.

Excepting for cases 8, 20, and 26, the *M. melitensis* was recovered from all cases examined where fever was present. When not found I think the prognosis is favourable for continued convalescence.

Urines.—The technique as recommended by Major Horrocks was followed—the penis being washed with carbolic acid solution, the urine collected in a sterile test-tube after a part had been passed to clear the urethra, and then 0·2 c.c. plated on litmus-glucose-nutrose-agar, incubated at 37° C.

In all 46 urines have been thus examined, of 18 patients, with the following results. In the great majority of the plates, in 24 hours the surface was covered by a spreading foul-smelling acid organism, or was thickly studded with opaque rapidly growing colonies of a rather large coccus, but in two instances typical colonies of *M. melitensis* were present as a pure culture, both being from the same patient.

| No. of case. | No. of examinations. | Condition. | Result. |
|--------------|----------------------|---------------------|--|
| 1 | 1 | Acute relapse | Negative |
| 2 | 7 | " " | " |
| 3 | 1 | " " T. 103 | " |
| 4 | 1 | " " | " |
| 5 | 2 | " " | " |
| 6 | 5 | " " | " |
| 7 | 3 | " " | " |
| 8 | 1 | Convalescent | " |
| 9 | 7 | " | " |
| 10 | 2 | Acute relapse | " |
| 11 | 2 | " " | " |
| 12 | 2 | " " | " |
| 13 | 1 | " " | " |
| 14 | 1 | " " | " |
| 15 | 4 | " " | " |
| 16 | 2 | " " | " |
| 17 | 11 | " " | { 1 with 16 col. on plate 1 with 14 col. on plate, pure culture of <i>M. melitensis</i> 9 rapidly overgrown |
| 18 | 1 | " " | |

Unless the urine is free from other organisms, there seemed to be little chance of isolating the *M. melitensis*, the growth of this organism being so much slower than most of the others present.

Case 16 was very acute, in a typhoid condition, and the urine was drawn off with a catheter, but was full of other organisms.

Sputum.—As several of the cases recently received into Haslar were suffering from bronchial catarrh, with expectoration of mucoid sputum, I thought it possible that in these one might be able to isolate the organism from the sputum.

Technique.—The sputum in the early morning was received into a sterile test-tube, a fragment of the thickest portion was then fished out on a platinum loop, thoroughly washed in a tube of sterilised water, and again removed to a second test-tube of sterile water, thoroughly mixed, and 0.2 c.c. plated out on nutrose-glucose-litmus-agar, incubated at 37°.

So far no colonies resembling the *M. melitensis* have been met with, and as all the cases have ceased to expectorate, the experiment has ceased.

98 *Isolation of M. Melitensis from the Blood, Urine, etc.*

| No. of case. | No. of experiment. | Condition. | Character of sputum. | Result. |
|-----------------|-----------------------|---------------------|-------------------------|----------|
| 1 | 1 | Acute relapse | Muco. purt. | Negative |
| | 2 | " " | " | " |
| 2 | 3 | Convalescent | Mucoid | " |
| 3 | 4 | Acute relapse | " | " |
| 4 | 5 | " " | " | " |
| | 6 | " " | " | " |
| 5 | 7 | " " | " | " |
| | 8 | " " | " | " |
| | 9 | " " | " | " |
| 6 | 10 | " " T. 104..... | " | " |

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